TITLE:

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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Dear editor,

Please find enclosed all the documents previously requested by the Journal of Hepatology: the original approved protocol, statistical analysis plan, and patient information sheet, and all subsequent amendments, and institutional review board approval from all participating centers.

No overall page numbering and no *Table of Content* have been generated as each document that comes with its own page numbering has been exported from PDF to Word as per requested formatting. All documents are presented in the following order of appearance.

- **Protocol (From version 1.0 dd. March 25th, 2016 to Version 6.1 dd. February 05th, 2019)**

All versions of the HEP101 protocols submitted and approved by the National Competent authorities and the Ethics Committee in Belgium, France, Spain and Bulgaria are enclosed.

Protocol Version	Date
Version 1.0	March 25th, 2016
Version 1.1	May 25th, 2016
Version 1.2	July 26th, 2016
Version 1.3	October 26th, 2016
Version 2.0	December 13th, 2016
Version 2.1	December 20th, 2016
Version 3.1	March 21st, 2017
Version 3.2	May 11th, 2017
Version 3.3	October 30th, 2017
Version 4.0	February 15th, 2018
Version 5.0	June 26th, 2018
Version 5.1	June 26th, 2018
Version 6.0	December 14th, 2018
Version 6.1	February 05th, 2019

Please note that the version 3.0 (dd. March 21st, 2017) has been submitted but not approved following questions and remarks raised by the Ethics Committee and the National Competent authorities.

- **Statistical Analysis Plan (SAP)**

The version 1.0 (dd. December 05th, 2018), the version 2.0 (dd. February 25th, 2019), the version 3.0 (dd. October 25th, 2019) and the version 4.0 (dd. November 26th, 2019) are enclosed. A Version 5.0 is currently under revision for the final database lock.

- **Informed Consent Form (ICF)**

All version of the HEP101 ICFs submitted and approved by the Ethics Committee in Belgium, France, Spain and Bulgaria are enclosed.

ICFs Version	Date
Version 1.1	June 28th, 2016
Version 1.2	September 12th, 2016
Version 1.3	October 25th, 2016
Version 1.4	October 27th, 2016
Version 1.5	November 24th, 2016
Version 2.1	March, 09th, 2018
Version 3.0	March 13th, 2018
Version 4.0	June 26th, 2018
Version 5.0	December 14th, 2018

Please note that the Version 1.0 (dd. April 07th, 2016) has been submitted but not approved following questions and remarks raised by the Ethics Committee. Please also note that the ICFs version 1.1, 1.2, 1.3, 1.4 and 1.5 are only available in French and Dutch. Indeed, these subsequent versions have been amended following remarks raised by the Belgium and French agencies, only the translation versions (French and Dutch) have been amended and approved.

- **Ethics Committee and Competent Authorities Approvals**

The HEP101 study has been fully approved in Belgium, France, Spain and Bulgaria. The approvals of the Ethics Committee and National Competent Authorities are enclosed.

In Belgium and Bulgaria, the study is submitted to the central Ethics Committee and the local ones (corresponding to each opened sites). However, the central Ethics Committee is responsible for providing the single opinion based on comments and questions raised by the locals Ethics Committee to the Central Ethics Committee. Therefore, no approval is received from the locals Ethics Committee.

In France and Spain, the HEP101 study is submitted to only one Central Ethics Committee. All participating sites are mentioned in the submitted dossier.

In Belgium, France, Spain & Bulgaria, the HEP101 study has been submitted to the Ethics Committee and the Competent Authorities in parallel.

I remain at your disposal if you have any questions or remarks, please do not hesitate to contact

me.

Sincerely yours,

Prof. Etienne Sokal

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 1.0 – 25 march 2016

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
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LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 1.0 - 24 march 2016
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Twelve (12) evaluable Patients
Number of Centers	5-10 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients. Study periods The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation.

	The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up. Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members. • When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data. • When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient. • When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

	Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period. After completion of this study, patients will be followed-up in the Product registry.
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50x106 cells/ml equivalent to 250x106 cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on patient's status. The infusion rate shall not exceed 1 ml per min. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 Billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion. In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 Billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before the first HepaStem infusion of the day.
Eligibility - Inclusion Criteria	Inclusion creteria: 1. Adult aged between 18 and 70 year old. 2. Informed Consent.

	N.B: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement. 3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan). 4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions ACLF grade 1 eligible subset: – liver failure plus cerebral and/or kidney dysfunction – renal failure plus cerebral dysfunction – cerebral failure plus kidney dysfunction – coagulation failure plus cerebral and/or kidney dysfunction Or ACLF grade 2 eligible subset: - Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure. Organ dysfuncytions or failures are defined according to CLIF-C OF score as below: Diagnostic criteria of kidney and cerebral dysfunction – kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL – cerebral: moderate impairment of brain function as defined by grade I-II Hepatic Encephalopathy based on West Haven criteria Diagnostic criteria of organ failures – liver: serum bilirubin ≥ 12 mg/dL – kidney: serum creatinine ≥ 2 mg/dL – cerebral: grade III-IV HE based on West Haven criteria – coagulation: international normalized ratio [INR] ≥ 2.5
Eligibility - Exclusion Criteria	Exclusion creteria: 1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam. 2. Known prothrombotic disease or medical history of thrombotic events. 3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h.

	4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 5. Clinical evidence of aspergilus infection. 6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria. 7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation. 8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion. 9. MELD score > 35. 10. Previous organ transplantation and/or ongoing immunosuppressive treatments. 11. Postoperative-decompensation following hepatectomy. 12. Renal failure due to chronic kidney disease. 13. Clinically significant left-right cardiac shunt. 14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions. 15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. 16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 18. Participation to any other interventional study within the last 4 weeks. 19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisouness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

	The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline.
	
	Secondary Endpoints:
	
	• Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
	  ○ Mortality
	  ○ Liver transplantation
	  ○ Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score.
	• Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
	  ○ Bilirubin, creatinine, INR and albumin values
	
	• Long term safety follow-up
	  ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1
	  ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation
	  ○ New ACLF episode will be summarized at Month 3 and Year 1

Study Assessment visits	Study visits During the screening and and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given. on HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be performed,

	as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.
	
	A study visit will be performed on Day 14 ± 2 days, including the evaluations listed below.
	
	On the other days during the hospital stay, patients will be followed-up according to usual practice.
	
	After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.
	
	After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).
	
	Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).
	
	At the Month 12 study visit, patients will be invited to be included in the Product Registry.
	
	<u>Study assessments</u>
	
	• All AEs up to Day 28
	• All AESI up to Year 1
	• Concomitant medication modifications up to Day 28
	• Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3. 6 and 12
	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ White Blood Cell count, Red Blood Cell count, platelets

	○ GOT, GPT, bilirubin, alkaline phosphatase, γ GT, ○ Creatinine, Urea or BUN ○ CRP ○ INR, aPTT ○ Serum albumin, sodium, potassium, • Lipase: at screening • Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission) • Urine test (Sediment, Creat, Glc, Protein, Albm): at screening • Protein C, Protein S, anti-thrombin III: at screening • Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab) • Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab) • Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab) • Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 • Chest x-ray, • Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected) • ECG: at screening (if not performed during same admission) • Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion • A transjugular liver biopsy: optional (if not already performed during the same admission) A SMC will review safety data and advice on study conduct. Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion. In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw. In case of liver transplantation, a sample of the explanted liver will be collected if possible.
Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).

Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics. Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who receive at least one infusion. All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

	AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The Report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Study Flow chart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	Baseline	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	D21[b]	D28[c]	M2	M3	M6	M12
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

ǂ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

1. BACKGROUND AND RATIONALE

1.1. ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Epidemiological studies indicate that there is an increasing prevalence of liver cirrhosis related to chronic infection by hepatitis C or B virus, alcohol consumption and non-alcoholic steatohepatitis worldwide (Murray *et al.* 2012). The natural course of cirrhosis is from compensated to decompensated disease. Decompensation is characterized by the development of major complications of liver disease (variceal bleeding, ascites, hepatic encephalopathy and bacterial infections) and is associated with poor prognosis. In addition to acute decompensation, ACLF is characterized by organ/system failure(s) (liver, kidney, brain, coagulation, circulation and/or lung) and high short-term mortality (33% at 28 days and 51% at 90 days). Approximately 31% of patients admitted to hospital for acute decompensation of cirrhosis present ACLF at admission (20%) or develop the syndrome during hospitalization (11%) (Moreau *et al.* 2013). Mortality rate depends on the number of failing organs as defined by the CLIF-SOFA score or the CLIF-OF score (a simplified version of the CLIF-SOFA score) (Table 1-1) (Moreau et al. 2013, Arroyo et al. 2015). Three grades define ACLF severity (Table 1-2). ACLF grade 1, defined as single kidney failure or single "non-kidney" organ failure with serum creatinine of 1.5-1.9 mg/dL and/or hepatic encephalopathy grade 1-2, is the most prevalent form of ACLF (15.8% of patients admitted at hospital with acute decompensation) and has a 28-day mortality rate of 23%. Patients with ACLF grade 2 (2 failing organs; prevalence 10.9%) have an intermediate prognosis (28-day mortality rate of 31%). Finally, ACLF grade 3 (with 3 or more organ failures) is the less frequent form of ACLF (4.4%) but shows extremely high mortality rates reaching 75% at 28 days.

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years, frequently alcoholics, in relation to a systemic inflammatory reaction due to bacterial infections, acute alcoholic liver injury or, in 40% of patients, to as yet unidentified precipitating events (Moreau et al. 2013). The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion, which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 or renal replacement
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by translocation of

bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may causes necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidences support the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory

disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properites of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013). Later, the technology of large-scale cell production was transferred to Promethera Biosciences which produce clinical batches of HHALPC in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs). In immunodeficient (SCID) mice transplanted with liver-derived progenitor cells, evidence of presence of human hepatocyte-like cells in the liver supported the biological plausibility of cell engraftment (Najimi *et al.* 2007; Khuu *et al.* 2011; Khuu *et al.* 2013). Short-term biodistribution assessed in rats using cells labeled with oxine ^{111}-Indium showed that cells concentrated in the liver (until 72 hours) (Tondreau et al. in preparation). Risk of tumor formation has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. *In vitro* studies showed that liver-derived progenitor cells present a low immunogenic phenotype (Sana *et al.* 2014). These cells have a pro-coagulant effect, similar to bone marrow derived MSCs, which may favor thrombosis. A study showed that concomitant treatment with an antithrombin activator or direct factor Xa inhibitor and direct thrombin inhibitor proved to be a particularly effective combination for controlling the procoagulant effects both *in vitro* and *in vivo* (Stephenne *et al.* 2012) (Please refer to the IB for more details).

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

Selected patients under the hospital exemption regulation were treated with liver-derived progenitor cells infused *via* the portal vein. In one of them, short-term biodistribution assessed using cells labeled with oxine ^{111}-Indium showed liver biodistribution of the cells (Sokal *et al.* 2013; Defresne *et al.* 2014).

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg) (4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in

cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. This data lays the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

A hospital exemption treatment was conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated well this cell infusion, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, right ankle, and spine, and had disappeared from the lungs.

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor

(HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For examples, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012). In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

The doses reported in publications on MSCs evaluation in immune-mediated diseases are varying usually between 0.5 to 3 millions cells/kg/infusion (Vandermeulen et al. 2014, Sharma et al. 2014). Single infusion or repeated infusions are reported. For example, remestemcel-L (Prochymal®, Mesoblast) was given IV at a dose of 2 x 10^6 MSCs/kg of body weight twice weekly for 4 consecutive weeks. Patients received all 8 infusions in the initial treatment plan by day 28. Infusions were administered at least 3 days apart (Kurtzberg et al. 2014). In some reports, higher doses were given, up to 800 millions cells/infusion (~11 millions/kg/infusion) (Ra et al. 2011, Mayer et al. 2013, Lublin et al. 2014, Melmed et al. 2015). In inflammatory liver diseases, doses varying between 0.5 to 5 millions cells have been reported (Berardis et

al. 2015). To be noted, the mode of administration was IV route and no concomitant administration of anti-coagulation medication was reported.

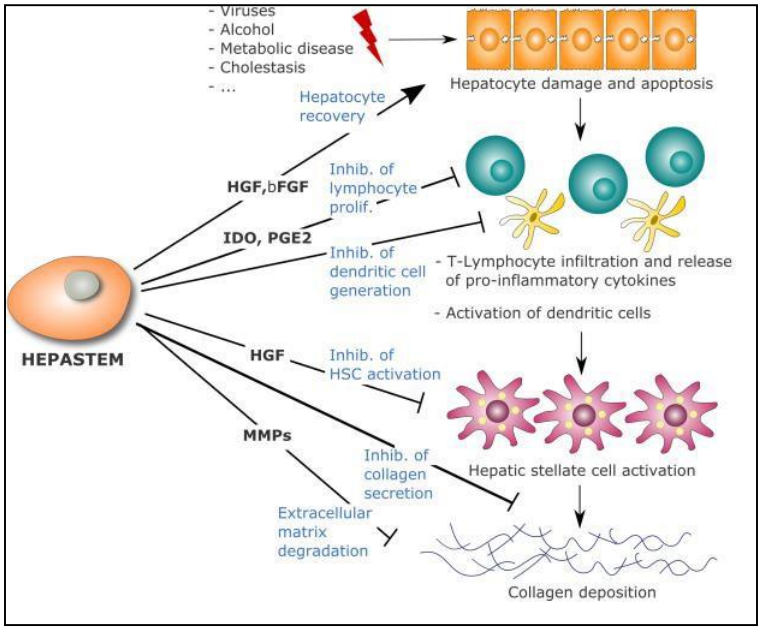
1.2.6. Pre-clinical immunomodulatory data of liver-derived progenitor cells

The first transcriptomics and secretomics tests performed on liver-derived progenitor cells grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models present important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.7. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing in the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.8. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts of 6 ACLF patients each. The study will include patients with ACLF grade 1 or 2 (excluding those with renal organ failure only, or those with circulatory or respiratory failure). It is planned to have a first group of 6 patients (cohort 1) being administered with 4 infusions of 250 millions cells each. Once this has been proven safe, a second group of 6 patients (cohort 2) will receive 4 infusions of 500 milions cells each. The infusions will be administered over 2 weeks with the first infusion started within a few days after patient's hospitalisation due to acute liver decompensation leading to ACLF. HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period.

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 6 patients, appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

HepaStem administration will be started rapidly after hospitalisation and will be completed within 2 weeks. This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of ACLF patients. As ACLF is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up for safety up 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining design of future efficacy clinical studies.

Study population

The selected adult population presents an expected mortality rate at short-term (28 days) close to 30%, which justify the use of a novel approach carrying potential short term benefits based on its immunomodulatory and anti-inflammatory properties. The short term mortality risk at 28 days is estimated close to 23% for ACLF grade 1 or 31% for ACLF grade 2. Patients with ACLF grade 1 with kidney failure only will be excluded as their mortality risk at 28 days is actually close (< 20%) to that of patients

with one (non-kidney) organ failure only (No ACLF) (Moreau et al. 2013). Patients with ACLF grade 3 will be excluded as they have a mortality rate reaching 75% at 28 days, making difficult to assess any safety or efficacy cell effect in this group. ACLF mainly occurs in cirrhotic patients aged around 50-60 years.

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 21 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 42 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 millions cells per infusion) is close to MSC doses given in other trials for immune mediated inflammatory diseases (Section 1.2.5), therefore, it is expected to show similar safety and efficacy profile. It corresponds also to the dose of liver-dirived progenitor cells administered via IV to the heamophila patient (see 1.2.4). The second selected dose represents a two-fold increase, still in the range of doses reported for MSCs. In addition, both doses are in the low range compared to HepaStem doses given in HEP001 paediatric study where administration of 500 millions cells per day was shown to be safe and well tolerated (see 1.2.3).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 UI/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damages nor any respiratory symptoms, before homing mainly in the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in ACLF patients, especially since it allows repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs have also a procoagulant activity comparable to liver-derived progenitor cells (Stephenne et al. 2012), nevertheless literature report

show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015). Furthermore, it has been established that hemostatic potential in patients with chronic liver disease is in a rebalanced status due to concomittant decrease in pro and anti-hemostatic drivers. In ACLF patients, the inflammatory process may trigger the unstable balance of hemostasis of cirrhotic patients to any of two states and may be manifested by either bleeding or thrombotic complications (Blasco-Algora et al. 2015). Thus an anti-coagulation may be contra-indicated in ACLF patients as they could be at risk of gastrointestinal hemorrhage, risks that may not be assessed by coagulation tests (prothombine time, INR, thrombin generation and thromboelastometry) (Lisman et al. 2012, Stravitz et al. 2012, Tripodi et al. 2009a, Tripodi et al. 2009b). Furthermore, bivalirudin use is not validated in cirrhotic patients. In order to mitigate risks of thrombosis in ACLF patients receiving HepaStem, several precautionary measures will be taken, as described in Section 5.5.1.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

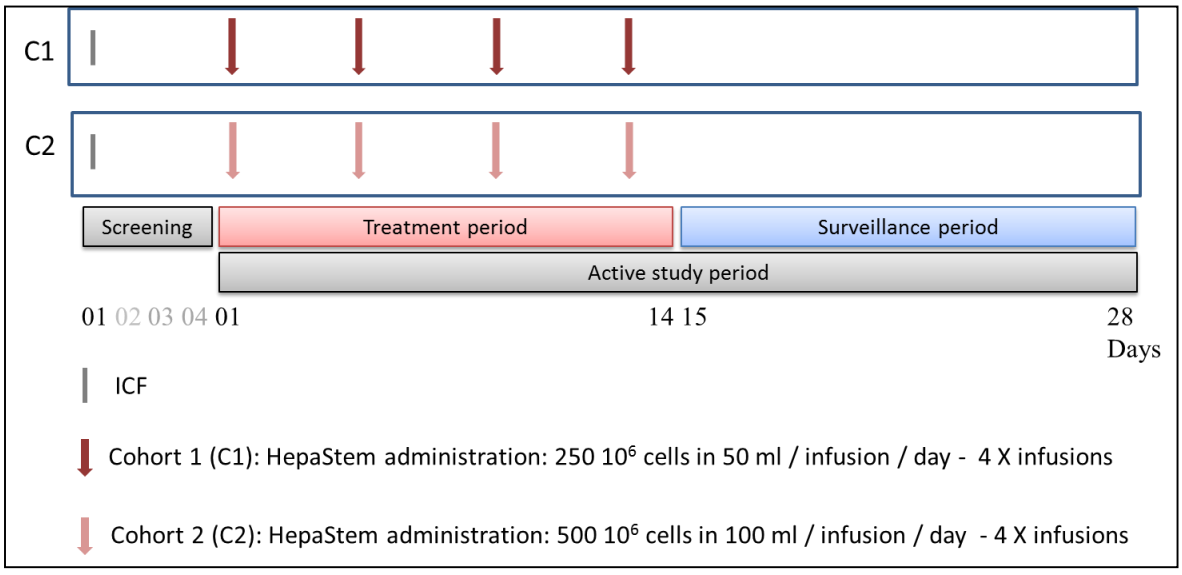
Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

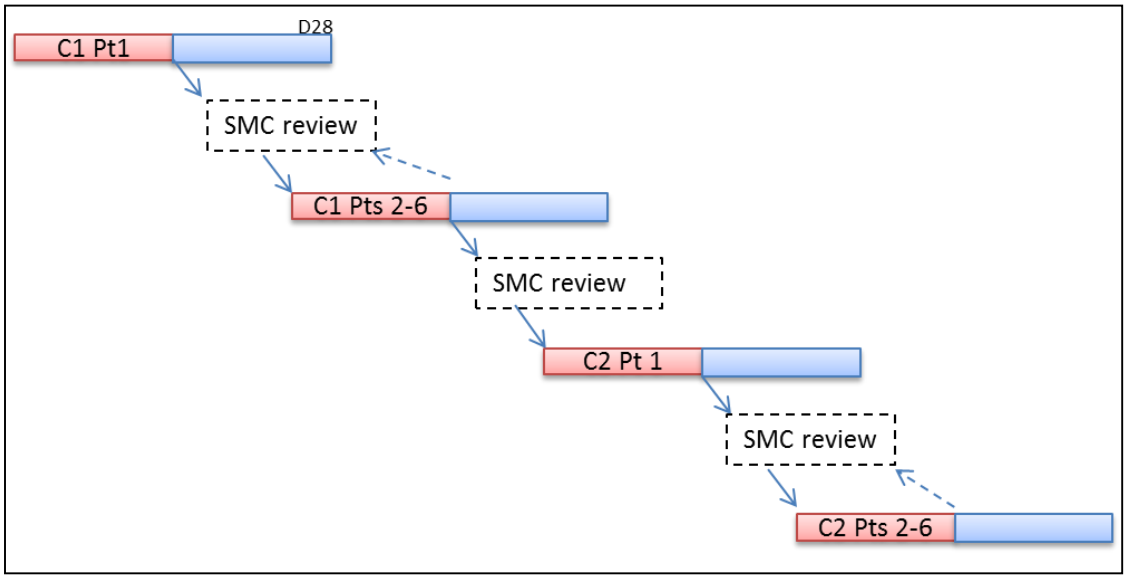
Figure 3-1 Study scheme of active study period



The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members (See Section 9.13):

- When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.
- When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient.
- When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring Committe



The study assessments are described in Section 6.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be followed-up in the Product registry.

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
  - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation
- New ACLF episode will be sumamrized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in 2 successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in 5 to 10 clinical centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days ($\pm$ 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Patients with ACLF at first evaluation post-admission or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
   N.B: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

   - liver histology or
   - clinical and imaging examination (may include fibroscan).

4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions

ACLF grade 1 eligible subset:

- liver **failure** plus cerebral and/or kidney **dysfunction**
- renal **failure** plus cerebral **dysfunction**
- cerebral **failure** plus kidney **dysfunction**
- coagulation **failure** plus cerebral and/or kidney **dysfunction**

Or

ACLF grade 2 eligible subset:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfuncytions or failures are defined according to CLIF-C OF score as below

Diagnostic criteria of kidney and cerebral dysfunction

- kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL.
- cerebral: moderate impairment of brain function as defined by grade I-II HE based on West Haven criteria.

Diagnostic criteria of organ failures

- liver: serum bilirubin ≥ 12 mg/dL;
- kidney: serum creatinine ≥ 2 mg/dL;
- cerebral: grade III-IV HE based on West Haven criteria;
- coagulation: international normalized ratio [INR] ≥ 2.5

For both grades, patients with circulatory and/or respiratory failure are excluded (see exclusion criteria 6 and 7).

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Known prothrombotic disease or medical history of thrombotic events.
3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
5. Clinical evidence of aspergilus infection.
6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria.
7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion.
9. MELD score > 35.
10. Previous organ transplantation and/or ongoing immunosuppressive treatments.
11. Postoperative-decompensation following hepatectomy.
12. Renal failure due to chronic kidney disease.
13. Clinically significant left-right cardiac shunt.

14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
18. Participation to any other interventional study within the last 4 weeks.
19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration are the following:

Transitory discontinuation:

- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem. Infusions may be restarted after recovery. If planned infusions are not performed within the 2 week treatment period (± 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAW CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee

- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No Patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. ACLF resolution or detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and-90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be

reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and the syringe will be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days.

In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes.

In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate or ICUs or standard units. Patients will be hospitalised during HepaStem treatment period.

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension have to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics and risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis, respiratory disorder as cells first transit to the lungs, hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the below mentioned risks, there might be other, at this time, unknown risks.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lungs disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect. **Number of cells administered per infusion will be maximum 500 millions cells and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min**. The lower dose regimen will be applied before the higher one. These doses are in the low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication (See Section 1).

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions.

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with medical history of thrombotic events or known prothrombotic disease will be excluded from the study.

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity

was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Long Term Safety follow-up Period of 1 year. Thereafter, they will be followed-up the the product registry.

Thereafter, patients will be followed in the Product registry.

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given.

on HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

A study visit will be performed on Day 14 ± 2 days, including the evaluations listed below.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be included in the Product Registry.

6.2.1. Study assessments

- All AEs up to Day 28
- All AESI up to Year 1
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets
    - GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening
- Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28
- Chest x-ray,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)

- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion
- A transjugular liver biopsy: optional (if not already performed during the same admission)

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

Thromboelastogram and thromboglobulin generation tests

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min. twice). The plasma will be colleted and put in cryotubes (min. 2 tubes containing each min. 500 microL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Hippocrate 10,
1200 Woluwe-Saint Lambert BELGIUM

Anti-HLA antibodies

Serum will be collected and frozen.

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Hippocrate 10 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHARTS

Table 6-1 Study Flowchart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	Baseline	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	D21[b]	D28[c]	M2	M3	M6	M12
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

‡ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome
- Liver Transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

Possibly

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probable

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definite

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

Moderate, when interfering with ADL;

Severe, when impeding with ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SAR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2.

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics.

Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated.

Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable

privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences must "verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection".

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator's Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient's further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients,

safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of Promethera members including medical monitor, pharmacovigilance representative, clinical representive and external members with expertise

in liver disease or other relevant medical fields. The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

20. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.
21. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
22. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
23. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.
    - When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.
    - When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient.
    - When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.
24. The SMC will review severe thrombotic events assessed as related to HepaStem administration by the investigator.
25. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- One or more biostatisticians
- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists may be invited to participate as non-voting members at any time if additional expertise is desired.

The frequency of SMC meetings will depend on several factors including the rate of enrollment, completion of five patients of the low dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL or renal replacement
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

Arroyo et al. 2015

11.2. MELD SCORE

MELD Score based on
- serum Creatinin
- serum Bilirubin and
- INR

Chung et al. 2012

11.3. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 25 March 2016

Version Number: 1.0

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____ _____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 25 March 2016

Version Number: 1.0

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competente Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

_____ _____

Joëlle Thonnard, Director Clinical and Medical Affairs Date (dd/mm/yyyy)

_____ _____

Etienne Sokal, MD Chief Innovation & Scientific Officer Date (dd/mm/yyyy)

_____ _____

John Tchelingerian, CEO Date (dd/mm/yyyy)

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 1.1 – 25 May 2016

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Innovation & Scientific Officer John Tchelingerian, MD – Chief Executive Officer Joëlle Thonnard, MD- Clinical & Medical Affairs Director
MEDICAL MONITOR	Joëlle Thonnard, T.+32.471.563.423
MONITORING & STUDY COORDINATION	Virginie Barthel, T.+32.471.784.097
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ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 1.1 – 25 May 2016
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Twelve (12) evaluable Patients
Number of Centers	5-10 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients. Study periods The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation. The study is divided in the following periods: screening period, active study period

	dived in treatment period and evaluation period, and long-term safety follow-up. Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members. • When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data. • When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized. • When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

	Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period. After completion of this study, patients will be followed-up in the Product registry.
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50x106 cells/ml equivalent to 250x106 cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on patient's status. The infusion rate shall not exceed 1 ml per min. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 Billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion. In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 Billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before the first HepaStem infusion of the day.
Eligibility - Inclusion Criteria	Inclusion creteria: 1. Adult aged between 18 and 70 year old. 2. Informed Consent. N.B: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if

	possible, after encephalopathy improvement. 3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan). 4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions <u>ACLF grade 1 eligible subset:</u> – liver failure plus cerebral and/or kidney dysfunction – renal failure plus cerebral dysfunction – cerebral failure plus kidney dysfunction – coagulation failure plus cerebral and/or kidney dysfunction <u>Or</u> <u>ACLF grade 2 eligible subset</u>: - Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure. Organ dysfuncytions or failures are defined according to CLIF-C OF score as below**:** <u>Diagnostic criteria of kidney and cerebral dysfunction</u> – kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL – cerebral: moderate impairment of brain function as defined by grade I-II Hepatic Encephalopathy based on West Haven criteria <u>Diagnostic criteria of organ failures</u> – liver: serum bilirubin ≥ 12 mg/dL – kidney: serum creatinine ≥ 2 mg/dL – cerebral: grade III-IV HE based on West Haven criteria – coagulation: international normalized ratio [INR] ≥ 2.5
Eligibility - Exclusion Criteria	<u>Exclusion creteria</u>: 1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam. 2. Known prothrombotic disease or medical history of thrombotic events. 3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h. 4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 5. Clinical evidence of aspergilus infection.

	6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria. 7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation. 8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion. 9. MELD score > 35. 10. Previous organ transplantation and/or ongoing immunosuppressive treatments. 11. Postoperative-decompensation following hepatectomy. 12. Renal failure due to chronic kidney disease. 13. Clinically significant left-right cardiac shunt. 14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions. 15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. 16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 18. Participation to any other interventional study within the last 4 weeks. 19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisouness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28. The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline. <u>Secondary Endpoints</u>: • Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1

	○ Mortality ○ Liver transplantation ○ Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score. • Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Bilirubin, creatinine, INR and albumin values • Long term safety follow-up ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1 ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation ○ New ACLF episode will be summarized at Month 3 and Year 1
Study Assessment visits	<u>Study visits</u> During the screening and and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given. on HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped. A study visit will be performed on Day 14 ± 2 days, including the evaluations listed below.

	On the other days during the hospital stay, patients will be followed-up according to usual practice.
	
	After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.
	
	After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).
	
	Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).
	
	At the Month 12 study visit, patients will be invited to be included in the Product Registry.
	
	<u>Study assessments</u>
	
	• All AEs up to Day 28
	• All AESI up to Year 1
	• Concomitant medication modifications up to Day 28
	• Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3. 6 and 12
	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ White Blood Cell count, Red Blood Cell count, platelets
	  ○ GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
	  ○ Creatinine, Urea or BUN
	  ○ CRP
	  ○ INR, aPTT

	○ Serum albumin, sodium, potassium, • Lipase: at screening • Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission) • Urine test (Sediment, Creat, Glc, Protein, Albm): at screening • Protein C, Protein S, anti-thrombin III: at screening • Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab) • Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab) • Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab) • Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 • Chest x-ray, • Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected) • ECG: at screening (if not performed during same admission) • Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion • A transjugular liver biopsy: – only data collection in case the biopsy has been done during the same admission – no specific transjugular liver biopsy required for the study protocol. A SMC will review safety data and advice on study conduct. Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion. In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw. In case of liver transplantation, a sample of the explanted liver will be collected if possible.
Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation

	medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics. Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who receive at least one infusion. All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor

	pharmacovigilance. The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The Report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Study Flow chart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	**Baseline**	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	**D21[b]**	**D28[c]**	**M2**	**M3**	**M6**	**M12**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

‡ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

1. BACKGROUND AND RATIONALE

1.1. ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Epidemiological studies indicate that there is an increasing prevalence of liver cirrhosis related to chronic infection by hepatitis C or B virus, alcohol consumption and non-alcoholic steatohepatitis worldwide (Murray *et al.* 2012). The natural course of cirrhosis is from compensated to decompensated disease. Decompensation is characterized by the development of major complications of liver disease (variceal bleeding, ascites, hepatic encephalopathy and bacterial infections) and is associated with poor prognosis. In addition to acute decompensation, ACLF is characterized by organ/system failure(s) (liver, kidney, brain, coagulation, circulation and/or lung) and high short-term mortality (33% at 28 days and 51% at 90 days). Approximately 31% of patients admitted to hospital for acute decompensation of cirrhosis present ACLF at admission (20%) or develop the syndrome during hospitalization (11%) (Moreau *et al.* 2013). Mortality rate depends on the number of failing organs as defined by the CLIF-SOFA score or the CLIF-OF score (a simplified version of the CLIF-SOFA score) (Table 1-1) (Moreau et al. 2013, Arroyo et al. 2015). Three grades define ACLF severity (Table 1-2). ACLF grade 1, defined as single kidney failure or single "non-kidney" organ failure with serum creatinine of 1.5-1.9 mg/dL and/or hepatic encephalopathy grade 1-2, is the most prevalent form of ACLF (15.8% of patients admitted at hospital with acute decompensation) and has a 28-day mortality rate of 23%. Patients with ACLF grade 2 (2 failing organs; prevalence 10.9%) have an intermediate prognosis (28-day mortality rate of 31%). Finally, ACLF grade 3 (with 3 or more organ failures) is the less frequent form of ACLF (4.4%) but shows extremely high mortality rates reaching 75% at 28 days.

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years, frequently alcoholics, in relation to a systemic inflammatory reaction due to bacterial infections, acute alcoholic liver injury or, in 40% of patients, to as yet unidentified precipitating events (Moreau et al. 2013). The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion, which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 or renal replacement
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by

translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may causes necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidences support the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety

profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properites of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013). Later, the technology of large-scale cell production was transferred to Promethera Biosciences which produce clinical batches of HHALPC in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs). In immunodeficient (SCID) mice transplanted with liver-derived progenitor cells, evidence of presence of human hepatocyte-like cells in the liver supported the biological plausibility of cell engraftment (Najimi *et al.* 2007; Khuu *et al.* 2011; Khuu *et al.* 2013). Short-term biodistribution assessed in rats using cells labeled with oxine ^{111}-Indium showed that cells concentrated in the liver (until 72 hours) (Tondreau et al. in preparation). Risk of tumor formation has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. *In vitro* studies showed that liver-derived progenitor cells present a low immunogenic phenotype (Sana *et al.* 2014). These cells have a pro-coagulant effect, similar to bone marrow derived MSCs, which may favor thrombosis. A study showed that concomitant treatment with an antithrombin activator or direct factor Xa inhibitor and direct thrombin inhibitor proved to be a particularly effective combination for controlling the procoagulant effects both *in vitro* and *in vivo* (Stephenne *et al.* 2012) (Please refer to the IB for more details).

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

Selected patients under the hospital exemption regulation were treated with liver-derived progenitor cells infused *via* the portal vein. In one of them, short-term biodistribution assessed using cells labeled with oxine ^{111}-Indium showed liver biodistribution of the cells (Sokal *et al.* 2013; Defresne *et al.* 2014).

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg)

(4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and

morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. This data lays the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

A hospital exemption treatment was conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated well this cell infusion, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, right ankle, and spine, and had disappeared from the lungs.

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell

activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For examples, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012). In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

The doses reported in publications on MSCs evaluation in immune-mediated diseases are varying usually between 0.5 to 3 millions cells/kg/infusion (Vandermeulen et al. 2014, Sharma et al. 2014). Single infusion or repeated infusions are reported. For example, remestemcel-L (Prochymal®, Osiris Therapeutics) was given IV at a dose of 2 x 10^6 MSCs/kg of body weight twice weekly for 4 consecutive weeks. Patients received all 8 infusions in the initial treatment plan by day 28. Infusions were administered at least 3 days apart (Kurtzberg et al. 2014). In some reports, higher doses were given, up

to 800 millions cells/infusion (~11 millions/kg/infusion) (Ra et al. 2011, Mayer et al. 2013, Lublin et al. 2014, Melmed et al. 2015). In inflammatory liver diseases, doses varying between 0.5 to 5 millions cells have been reported (Berardis et al. 2015). To be noted, the mode of administration was IV route and no concomitant administration of anti-coagulation medication was reported.

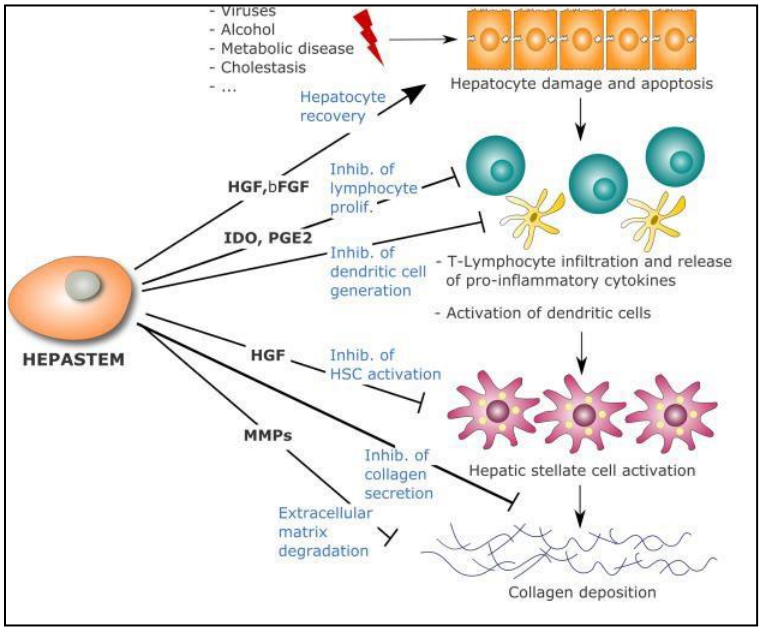
1.2.6. Pre-clinical immunomodulatory data of liver-derived progenitor cells

The first transcriptomics and secretomics tests performed on liver-derived progenitor cells grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models present important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.7. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing in the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.8. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts of 6 ACLF patients each. The study will include patients with ACLF grade 1 or 2 (excluding those with renal organ failure only, or those with circulatory or respiratory failure). It is planned to have a first group of 6 patients (cohort 1) being administered with 4 infusions of 250 millions cells each. Once this has been proven safe, a second group of 6 patients (cohort 2) will receive 4 infusions of 500 milions cells each. The infusions will be administered over 2 weeks with the first infusion started within a few days after patient's hospitalisation due to acute liver decompensation leading to ACLF. HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period.

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 6 patients, appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

HepaStem administration will be started rapidly after hospitalisation and will be completed within 2 weeks. This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of ACLF patients. As ACLF is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up for safety up 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining design of future efficacy clinical studies.

Study population

The selected adult population presents an expected mortality rate at short-term (28 days) close to 30%, which justify the use of a novel approach carrying potential short term benefits based on its immunomodulatory and anti-inflammatory properties. The short term mortality risk at 28 days is estimated close to 23% for ACLF grade 1 or 31% for ACLF grade 2. Patients with ACLF grade 1 with kidney

failure only will be excluded as their mortality risk at 28 days is actually close (< 20%) to that of patients with one (non-kidney) organ failure only (No ACLF) (Moreau et al. 2013). Patients with ACLF grade 3 will be excluded as they have a mortality rate reaching 75% at 28 days, making difficult to assess any safety or efficacy cell effect in this group. ACLF mainly occurs in cirrhotic patients aged around 50-60 years.

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 21 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 42 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 millions cells per infusion) is close to MSC doses given in other trials for immune mediated inflammatory diseases (Section 1.2.5), therefore, it is expected to show similar safety and efficacy profile. It corresponds also to the dose of liver-dirived progenitor cells administered via IV to the heamophila patient (see 1.2.4). The second selected dose represents a two-fold increase, still in the range of doses reported for MSCs. In addition, both doses are in the low range compared to HepaStem doses given in HEP001 paediatric study where administration of 500 millions cells per day was shown to be safe and well tolerated (see 1.2.3).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 UI/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damages nor any respiratory symptoms, before homing mainly in the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in ACLF patients, especially since it allows repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs have also a

procoagulant activity comparable to liver-derived progenitor cells (Stephenne et al. 2012), nevertheless literature report show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015). Furthermore, it has been established that hemostatic potential in patients with chronic liver disease is in a rebalanced status due to concomittant decrease in pro and anti-hemostatic drivers. In ACLF patients, the inflammatory process may trigger the unstable balance of hemostasis of cirrhotic patients to any of two states and may be manifested by either bleeding or thrombotic complications (Blasco-Algora et al. 2015). Thus an anti-coagulation may be contra-indicated in ACLF patients as they could be at risk of gastrointestinal hemorrhage, risks that may not be assessed by coagulation tests (prothombine time, INR, thrombin generation and thromboelastometry) (Lisman et al. 2012, Stravitz et al. 2012, Tripodi et al. 2009a, Tripodi et al. 2009b). Furthermore, bivalirudin use is not validated in cirrhotic patients. In order to mitigate risks of thrombosis in ACLF patients receiving HepaStem, several precautionary measures will be taken, as described in Section 5.5.1.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

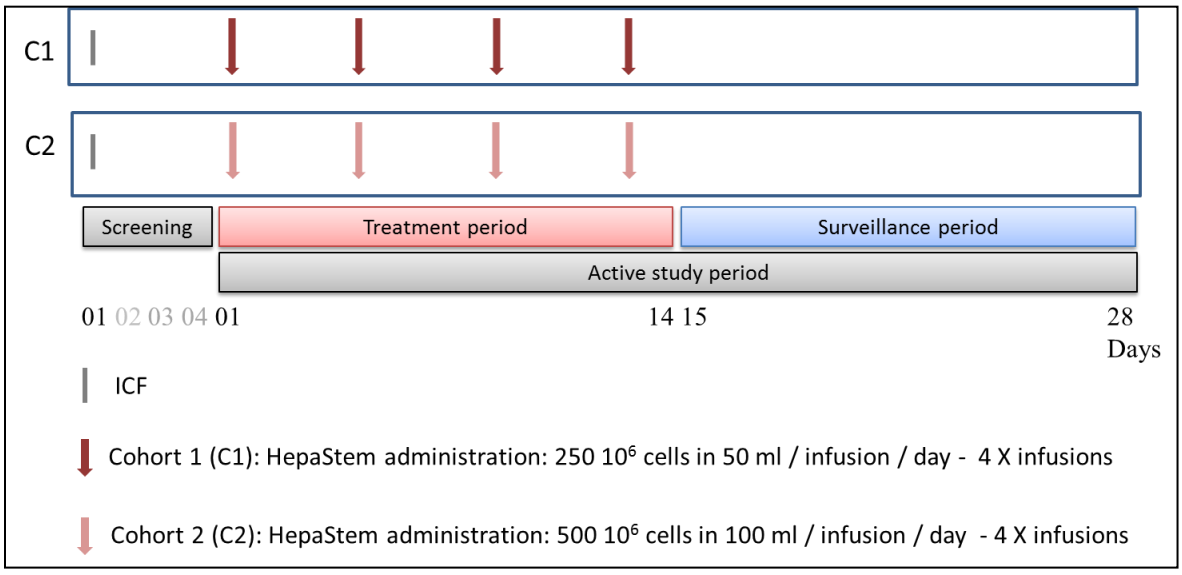
Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



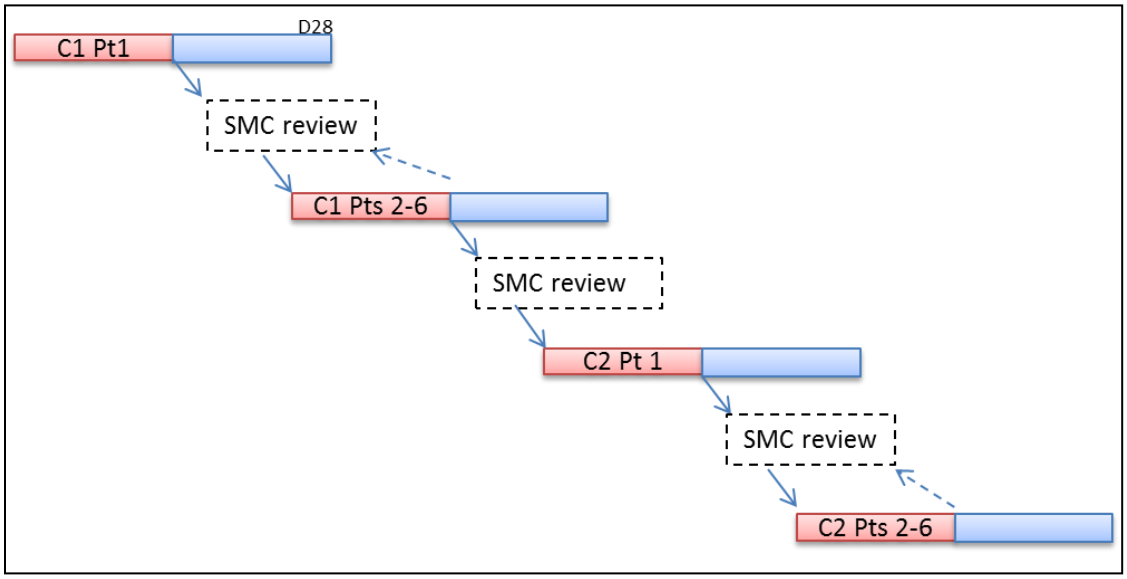
The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members (See Section 9.13):

- When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

- When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

- When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring Committe



The study assessments are described in Section 6.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be followed-up in the Product registry.

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
  - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation
- New ACLF episode will be sumamrized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in 2 successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in 5 to 10 clinical centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Patients with ACLF at first evaluation post-admission or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
   N.B: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

   - liver histology or
   - clinical and imaging examination (may include fibroscan).

4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions

ACLF grade 1 eligible subset:

- liver **failure** plus cerebral and/or kidney **dysfunction**
- renal **failure** plus cerebral **dysfunction**
- cerebral **failure** plus kidney **dysfunction**
- coagulation **failure** plus cerebral and/or kidney **dysfunction**

Or

ACLF grade 2 eligible subset:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfuncytions or failures are defined according to CLIF-C OF score as below

Diagnostic criteria of kidney and cerebral dysfunction

- kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL.
- cerebral: moderate impairment of brain function as defined by grade I-II HE based on West Haven criteria.

Diagnostic criteria of organ failures

- liver: serum bilirubin ≥ 12 mg/dL;
- kidney: serum creatinine ≥ 2 mg/dL;
- cerebral: grade III-IV HE based on West Haven criteria;
- coagulation: international normalized ratio [INR] ≥ 2.5

For both grades, patients with circulatory and/or respiratory failure are excluded (see exclusion criteria 6 and 7).

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Known prothrombotic disease or medical history of thrombotic events.
3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
5. Clinical evidence of aspergilus infection.
6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria.
7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion.
9. MELD score > 35.
10. Previous organ transplantation and/or ongoing immunosuppressive treatments.
11. Postoperative-decompensation following hepatectomy.
12. Renal failure due to chronic kidney disease.
13. Clinically significant left-right cardiac shunt.

14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
18. Participation to any other interventional study within the last 4 weeks.
19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration are the following:

Transitory discontinuation:

- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem. Infusions may be restarted after recovery. If planned infusions are not performed within the 2 week treatment period (± 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAW CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee

- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No Patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. ACLF resolution or detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and

the syringe will be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days.

In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes.

In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate or ICUs or standard units. Patients will be hospitalised during HepaStem treatment period.

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension have to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics and risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis, respiratory disorder as cells first transit to the lungs, hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the below mentioned risks, there might be other, at this time, unknown risks.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lungs disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect. **Number of cells administered per infusion will be maximum 500 millions cells and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min**. The lower dose regimen will be applied before the higher one. These doses are in the low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication (See Section 1).

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions.

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with medical history of thrombotic events or known prothrombotic disease will be excluded from the study.

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity

was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Long Term Safety follow-up Period of 1 year. Thereafter, they will be followed-up the the product registry.

Thereafter, patients will be followed in the Product registry.

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given.

on HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

A study visit will be performed on Day 14 ± 2 days, including the evaluations listed below.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be included in the Product Registry.

6.2.1. Study assessments

- All AEs up to Day 28
- All AESI up to Year 1
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets
    - GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening
- Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28
- Chest x-ray,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)

- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion
- A transjugular liver biopsy: – only data collection in case the biopsy has been done during the same admission – no specific transjugular liver biopsy required for the study protocol.

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

Thromboelastogram and thromboglobulin generation tests

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min. twice). The plasma will be colleted and put in cryotubes (min. 2 tubes containing each min. 500 microL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Hippocrate 10,
1200 Woluwe-Saint Lambert BELGIUM

Anti-HLA antibodies

Serum will be collected and frozen.

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Hippocrate 10 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHARTS

Table 6-1 Study Flowchart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	Baseline	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	D21[b]	D28[c]	M2	M3	M6	M12
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

‡ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome
- Liver Transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

Possibly

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probable

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definite

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

Moderate, when interfering with ADL;

Severe, when impeding with ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SAR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2.

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics.

Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences must "verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection".

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator's Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient's further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission

for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of Promethera members including medical monitor, pharmacovigilance representative, clinical representive and external members with expertise in liver disease or other relevant medical fields. The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

20. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.
21. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
22. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
23. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.
    - When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.
    - When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.
    - When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.
24. The SMC will review severe thrombotic events assessed as related to HepaStem administration by the investigator.

25. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- One or more biostatisticians
- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists may be invited to participate as non-voting members at any time if additional expertise is desired.

The frequency of SMC meetings will depend on several factors including the rate of enrollment, completion of five patients of the low dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC.

10. REFERENCE LIST

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL or renal replacement
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2	>300	≤300 - > 200	≤200
or SpO2/FiO2	>357	>214- ≤357	≤214

Arroyo et al. 2015

11.2. MELD SCORE

MELD Score based on
- serum Creatinin
- serum Bilirubin and
- INR

Chung et al. 2012

11.3. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 25 May 2016

Version Number: 1.1

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____

_____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 25 May 2016

Version Number: 1.1

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competente Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

_____ _____

Joëlle Thonnard, Director Clinical and Medical Affairs Date (dd/mm/yyyy)

_____ _____

Etienne Sokal, MD Chief Innovation & Scientific Officer Date (dd/mm/yyyy)

_____ _____

John Tchelingerian, CEO Date (dd/mm/yyyy)

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 1.2 – 20 July 2016

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Innovation & Scientific Officer John Tchelingerian, MD – Chief Executive Officer Joëlle Thonnard, MD- Clinical & Medical Affairs Director
MEDICAL MONITOR	Joëlle Thonnard, T.+32.471.563.423
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LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 1.2_20 July 2016
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Twelve (12) evaluable Patients
Number of Centers	5-10 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients. Study periods The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation. The study is divided in the following periods: screening period, active study period

	dived in treatment period and evaluation period, and long-term safety follow-up. Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members. • When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data. • When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advise on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized. • When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data.

	Furthermore, the 3 first patients of each cohort will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete sheme or premature stop).Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period. After completion of this study, patients will be invited to be followed-up in the long term safety follow up study (4 years).
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and thereafter for an additional period of 1 year in the long term safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at $50x10^6$ cells/ml equivalent to $250x10^6$ cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on patient's status. The infusion rate shall not exceed 1 ml per min. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 Billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion. In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 Billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before the first HepaStem infusion of the day.

<table>
<tr><td>Eligibility - Inclusion Criteria</td><td>
<u>Inclusion criteria</u>:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
<u>N.B:</u> In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan).

4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions

<u>ACLF grade 1 eligible subset:</u>

– liver failure plus cerebral and/or kidney dysfunction
– renal failure plus cerebral dysfunction
– cerebral failure plus kidney dysfunction
– coagulation failure plus cerebral and/or kidney dysfunction

<u>Or</u>

<u>ACLF grade 2 eligible subset</u>:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfuncytions or failures are defined according to CLIF-C OF score as below<u>:</u>

<u>Diagnostic criteria of kidney and cerebral dysfunction</u>

– kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL
– cerebral: moderate impairment of brain function as defined by grade I-II Hepatic Encephalopathy based on West Haven criteria

<u>Diagnostic criteria of organ failures</u>

– liver: serum bilirubin ≥ 12 mg/dL
– kidney: serum creatinine ≥ 2 mg/dL
– cerebral: grade III-IV HE based on West Haven criteria
– coagulation: international normalized ratio [INR] ≥ 2.5
</td></tr>
<tr><td>Eligibility - Exclusion Criteria</td><td>
<u>Exclusion criteria</u>:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Known prothrombotic disease or medical history of thrombotic events.
</td></tr>
</table>

	3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h. 4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 5. Clinical evidence of aspergilus infection. 6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria. 7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation. 8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion. 9. MELD score > 35. 10. Previous organ transplantation and/or ongoing immunosuppressive treatments. 11. Postoperative-decompensation following hepatectomy. 12. Renal failure due to chronic kidney disease. 13. Clinically significant left-right cardiac shunt. 14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions. 15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. 16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 18. Participation to any other interventional study within the last 4 weeks. 19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisouness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

	The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline.
	
	Secondary Endpoints:
	
	• Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
	  ○ Mortality
	  ○ Liver transplantation
	  ○ Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score.
	• Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
	  ○ Bilirubin, creatinine, INR and albumin values
	
	• Long term safety follow-up
	  ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1
	  ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of transplantation
	  ○ New ACLF episode will be summarized at Month 3 and Year 1

Study Assessment visits	Study visits During the screening and and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given. on HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be

	performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.
	
	A study visit will be performed on Day 14 ± 2 days, including the evaluations listed below.
	
	On the other days during the hospital stay, patients will be followed-up according to usual practice.
	
	After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.
	
	After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).
	
	Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).
	
	At the Month 12 study visit, patients will be invited to be included in the long term safety follow up study (4 years).
	
	<u>Study assessments</u>
	
	• All AEs up to Day 28
	• All AESI up to Year 1
	• Concomitant medication modifications up to Day 28
	• Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3, 6 and 12
	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.

	○ White Blood Cell count, Red Blood Cell count, platelets ○ GOT, GPT, bilirubin, alkaline phosphatase, γ GT, ○ Creatinine, Urea or BUN ○ CRP ○ INR, aPTT ○ Serum albumin, sodium, potassium, • Lipase: at screening • Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission) • Urine test (Sediment, Creat, Glc, Protein, Albm): at screening • Protein C, Protein S, anti-thrombin III: at screening • Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab) • Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab) • Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab) • Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 • Chest x-ray, • Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected) • ECG: at screening (if not performed during same admission) • Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion Transjugular liver biopsy: – only data collection in case the biopsy has been done during the same admission or previously – no transjugular liver biopsy is required for the study protocol.A SMC will review safety data and advice on study conduct. Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion. In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw. In case of liver transplantation, a sample of the explanted liver will be collected if possible.
Prohibited	Patients are requested to accept abstinence from alcohol during the active study

Medications and Food	period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics. Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who receive at least one infusion. All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study

	investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The Report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Study Flow chart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	**Baseline**	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	**D21[b]**	**D28[c]**	**M2**	**M3**	**M6**	**M12**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

‡ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

1. BACKGROUND AND RATIONALE

1.1. ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Epidemiological studies indicate that there is an increasing prevalence of liver cirrhosis related to chronic infection by hepatitis C or B virus, alcohol consumption and non-alcoholic steatohepatitis worldwide (Murray *et al.* 2012). The natural course of cirrhosis is from compensated to decompensated disease. Decompensation is characterized by the development of major complications of liver disease (variceal bleeding, ascites, hepatic encephalopathy and bacterial infections) and is associated with poor prognosis. In addition to acute decompensation, ACLF is characterized by organ/system failure(s) (liver, kidney, brain, coagulation, circulation and/or lung) and high short-term mortality (33% at 28 days and 51% at 90 days). Approximately 31% of patients admitted to hospital for acute decompensation of cirrhosis present ACLF at admission (20%) or develop the syndrome during hospitalization (11%) (Moreau *et al.* 2013). Mortality rate depends on the number of failing organs as defined by the CLIF-SOFA score or the CLIF-OF score (a simplified version of the CLIF-SOFA score) (Table 1-1) (Moreau et al. 2013, Arroyo et al. 2015). Three grades define ACLF severity (Table 1-2). ACLF grade 1, defined as single kidney failure or single "non-kidney" organ failure with serum creatinine of 1.5-1.9 mg/dL and/or hepatic encephalopathy grade 1-2, is the most prevalent form of ACLF (15.8% of patients admitted at hospital with acute decompensation) and has a 28-day mortality rate of 23%. Patients with ACLF grade 2 (2 failing organs; prevalence 10.9%) have an intermediate prognosis (28-day mortality rate of 31%). Finally, ACLF grade 3 (with 3 or more organ failures) is the less frequent form of ACLF (4.4%) but shows extremely high mortality rates reaching 75% at 28 days.

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years, frequently alcoholics, in relation to a systemic inflammatory reaction due to bacterial infections, acute alcoholic liver injury or, in 40% of patients, to as yet unidentified precipitating events (Moreau et al. 2013). The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion, which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 or renal replacement
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by

translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may causes necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidences support the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety

profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properites of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013). Later, the technology of large-scale cell production was transferred to Promethera Biosciences which produce clinical batches of HHALPC in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs). In immunodeficient (SCID) mice transplanted with liver-derived progenitor cells, evidence of presence of human hepatocyte-like cells in the liver supported the biological plausibility of cell engraftment (Najimi *et al.* 2007; Khuu *et al.* 2011; Khuu *et al.* 2013). Short-term biodistribution assessed in rats using cells labeled with oxine ^{111}-Indium showed that cells concentrated in the liver (until 72 hours) (Tondreau et al. in preparation). Risk of tumor formation has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. *In vitro* studies showed that liver-derived progenitor cells present a low immunogenic phenotype (Sana *et al.* 2014). These cells have a pro-coagulant effect, similar to bone marrow derived MSCs, which may favor thrombosis. A study showed that concomitant treatment with an antithrombin activator or direct factor Xa inhibitor and direct thrombin inhibitor proved to be a particularly effective combination for controlling the procoagulant effects both *in vitro* and *in vivo* (Stephenne *et al.* 2012) (Please refer to the IB for more details).

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

Selected patients under the hospital exemption regulation were treated with liver-derived progenitor cells infused *via* the portal vein. In one of them, short-term biodistribution assessed using cells labeled with oxine ^{111}-Indium showed liver biodistribution of the cells (Sokal *et al.* 2013; Defresne *et al.* 2014).

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg)

(4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and

morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. This data lays the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

A hospital exemption treatment was conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated well this cell infusion, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, right ankle, and spine, and had disappeared from the lungs.

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell

activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For examples, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012). In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

The doses reported in publications on MSCs evaluation in immune-mediated diseases are varying usually between 0.5 to 3 millions cells/kg/infusion (Vandermeulen et al. 2014, Sharma et al. 2014). Single infusion or repeated infusions are reported. For example, remestemcel-L (Prochymal®, Osiris Therapeutics) was given IV at a dose of 2 x 10^6 MSCs/kg of body weight twice weekly for 4 consecutive weeks. Patients received all 8 infusions in the initial treatment plan by day 28. Infusions were administered at least 3 days apart (Kurtzberg et al. 2014). In some reports, higher doses were given, up

to 800 millions cells/infusion (~11 millions/kg/infusion) (Ra et al. 2011, Mayer et al. 2013, Lublin et al. 2014, Melmed et al. 2015). In inflammatory liver diseases, doses varying between 0.5 to 5 millions cells have been reported (Berardis et al. 2015). To be noted, the mode of administration was IV route and no concomitant administration of anti-coagulation medication was reported.

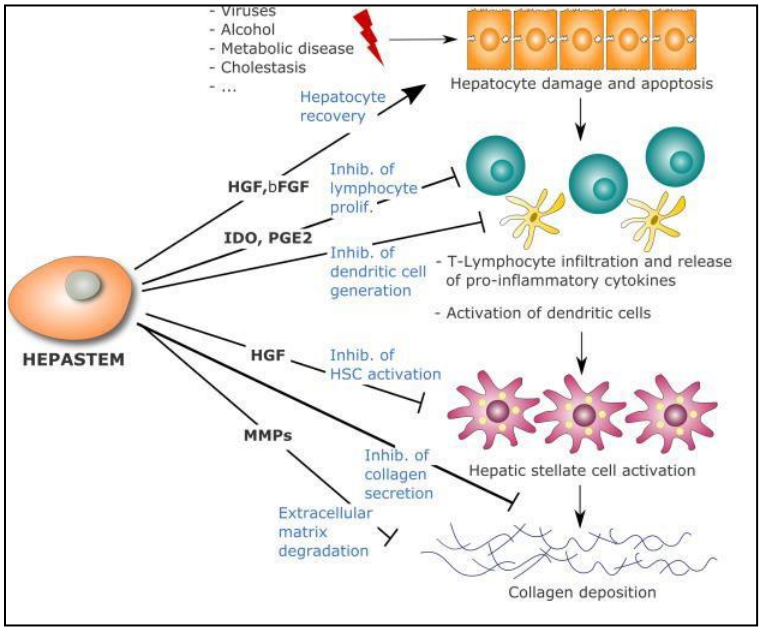
1.2.6. Pre-clinical immunomodulatory data of liver-derived progenitor cells

The first transcriptomics and secretomics tests performed on liver-derived progenitor cells grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models present important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.7. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing in the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.8. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts of 6 ACLF patients each. The study will include patients with ACLF grade 1 or 2 (excluding those with renal organ failure only, or those with circulatory or respiratory failure). It is planned to have a first group of 6 patients (cohort 1) being administered with 4 infusions of 250 millions cells each. Once this has been proven safe, a second group of 6 patients (cohort 2) will receive 4 infusions of 500 milions cells each. The infusions will be administered over 2 weeks with the first infusion started within a few days after patient's hospitalisation due to acute liver decompensation leading to ACLF. HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period.

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 6 patients, appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

HepaStem administration will be started rapidly after hospitalisation and will be completed within 2 weeks. This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of ACLF patients. As ACLF is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up for safety up 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining design of future efficacy clinical studies.

Study population

The selected adult population presents an expected mortality rate at short-term (28 days) close to 30%, which justify the use of a novel approach carrying potential short term benefits based on its immunomodulatory and anti-inflammatory properties. The short term mortality risk at 28 days is estimated close to 23% for ACLF grade 1 or 31% for ACLF grade 2. Patients with ACLF grade 1 with kidney

failure only will be excluded as their mortality risk at 28 days is actually close (< 20%) to that of patients with one (non-kidney) organ failure only (No ACLF) (Moreau et al. 2013). Patients with ACLF grade 3 will be excluded as they have a mortality rate reaching 75% at 28 days, making difficult to assess any safety or efficacy cell effect in this group. ACLF mainly occurs in cirrhotic patients aged around 50-60 years.

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 14 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 28 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 millions cells per infusion) is close to MSC doses given in other trials for immune mediated inflammatory diseases (Section 1.2.5), therefore, it is expected to show similar safety and efficacy profile. It corresponds also to the dose of liver-dirived progenitor cells administered via IV to the heamophila patient (see 1.2.4). The second selected dose represents a two-fold increase, still in the range of doses reported for MSCs. In addition, both doses are in the low range compared to HepaStem doses given in HEP001 paediatric study where administration of 500 millions cells per day was shown to be safe and well tolerated (see 1.2.3).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 UI/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damages nor any respiratory symptoms, before homing mainly in the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in ACLF patients, especially since it allows repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs have also a

procoagulant activity comparable to liver-derived progenitor cells (Stephenne et al. 2012), nevertheless literature report show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015). Furthermore, it has been established that hemostatic potential in patients with chronic liver disease is in a rebalanced status due to concomittant decrease in pro and anti-hemostatic drivers. In ACLF patients, the inflammatory process may trigger the unstable balance of hemostasis of cirrhotic patients to any of two states and may be manifested by either bleeding or thrombotic complications (Blasco-Algora et al. 2015). Thus an anti-coagulation may be contra-indicated in ACLF patients as they could be at risk of gastrointestinal hemorrhage, risks that may not be assessed by coagulation tests (prothombine time, INR, thrombin generation and thromboelastometry) (Lisman et al. 2012, Stravitz et al. 2012, Tripodi et al. 2009a, Tripodi et al. 2009b). Furthermore, bivalirudin use is not validated in cirrhotic patients. In order to mitigate risks of thrombosis in ACLF patients receiving HepaStem, several precautionary measures will be taken, as described in Section 5.5.1.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

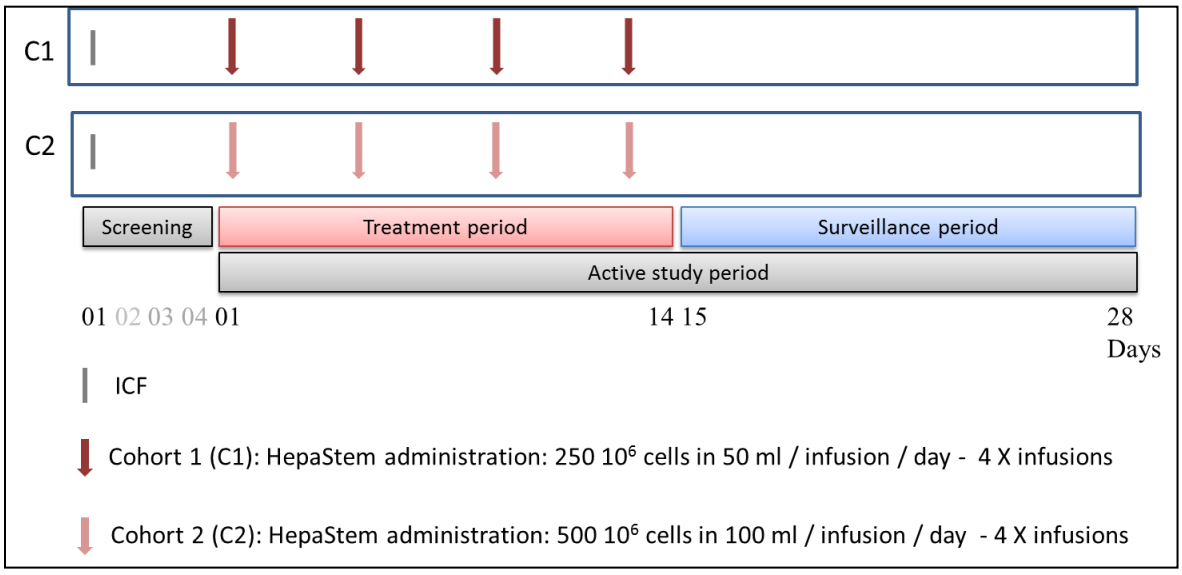
Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



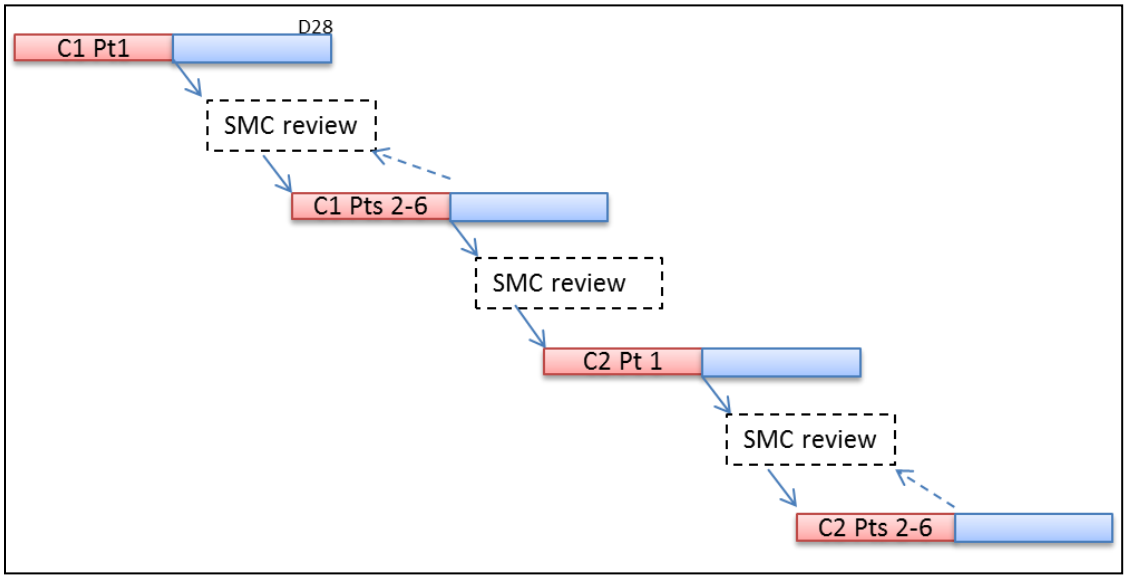
The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members (See Section 9.13):

- When the first cohort 1 evaluable patient will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

- When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

- When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring Committe



The study assessments are described in Section 6.

Furthermore, the 3 first patients of each cohort will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete scheme or premature stop). The sequential approach will be under the control of Promethera (based on review of eligibility criteria by the medical monitor and HepaStem delivery). In case of safety signal, the SMC will be involved in the AEs review and evaluation, and the SMC will advise on further inclusion.

These measures (SMC meetings and sequential treatment for the 3 first patients in each cohort) will allow respecting the progress of dose levels with limited risk for the patients.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be invited to be followed-up in the long term safety follow up study (4 years).

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
  - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of transplantation
- New ACLF episode will be summarized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in 2 successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in 5 to 10 clinical centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. The hospitalisation unit will be adapted according to the medical status of the patient and the organization of study center hospital. Patients with a low CLIF-OF score will be more likely included in the hepatology department (standard or intermediate care unit), while the patient with high CLIF-OF score will more likely be included in the Intensive Care Unit. Patients will remain hospitalised at least during the treatment period.

Patients with ACLF at first evaluation post-admission or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
   N.B.: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

   - liver histology or
   - clinical and imaging examination (may include fibroscan).

4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions

ACLF grade 1 eligible subset:

- liver **failure** plus cerebral and/or kidney **dysfunction**
- renal **failure** plus cerebral **dysfunction**
- cerebral **failure** plus kidney **dysfunction**
- coagulation **failure** plus cerebral and/or kidney **dysfunction**

Or

ACLF grade 2 eligible subset:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfuncytions or failures are defined according to CLIF-C OF score as below

Diagnostic criteria of kidney and cerebral dysfunction

- kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL.
- cerebral: moderate impairment of brain function as defined by grade I-II HE based on West Haven criteria.

Diagnostic criteria of organ failures

- liver: serum bilirubin ≥ 12 mg/dL;
- kidney: serum creatinine ≥ 2 mg/dL;
- cerebral: grade III-IV HE based on West Haven criteria;
- coagulation: international normalized ratio [INR] ≥ 2.5

For both grades, patients with circulatory and/or respiratory failure are excluded (see exclusion criteria 6 and 7).

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Known prothrombotic disease or medical history of thrombotic events.
3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
5. Clinical evidence of aspergilus infection.
6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria.
7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion.
9. MELD score > 35.

10. Previous organ transplantation and/or ongoing immunosuppressive treatments.
11. Postoperative-decompensation following hepatectomy.
12. Renal failure due to chronic kidney disease.
13. Clinically significant left-right cardiac shunt.
14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
18. Participation to any other interventional study within the last 4 weeks.
19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration for a patient are the following:

Transitory discontinuation:

- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem. Infusions may be restarted after recovery. If planned infusions are not performed within the 2 week treatment period (± 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, *i.e.*, thrombosis, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock, severe worsening of hepatic function.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAW CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No Patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. ACLF resolution or detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and

the syringe will be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days.

In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes.

In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate or ICUs or standard units, depending on the medical status of the patient and the organisation of study center hospital. Regardless of the unit of hospitalization, patients will remain hospitalised at least during the treatment period, with a close monitoring of each patient. During HepaStem infusion, a continuous monitoring of the vital signs of the patient is required.

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension have to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics and risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis, respiratory disorder as cells first transit to the lungs, hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the below mentioned risks, there might be other, at this time, unknown risks.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lungs disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect. **Number of cells administered per infusion will be maximum 500 millions cells and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min**. The lower dose regimen will be applied before the higher one. These doses are in the low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication (See Section 1).

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before,**

during and after each HepaStem infusion for all the patients based on vital signs (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions. Any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with medical history of thrombotic events or known prothrombotic disease will be excluded from the study.

In case of clinical signs or suspicion of pulmonary embolism, the investigator should perform exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigraphy.

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Long Term Safety follow-up Period of 1 year. Thereafter, patients will be invited to be followed-up in a long term safety follow up study (4 years).

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and and treatment period, patients will be hospitalised in intermediate or ICUs or standard units, depending of the severity of the patient disease. Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given.

On HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

During the infusion, the patient will be continuously monitored for early detection of any potential AEs.

A study visit will be performed on Day 14 ± 2 days, including the evaluations listed below.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

In case of any suspicion of AE, the investigator will perform the exams she/he evaluates as appropriate. In particular, any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement. In case of clinical signs or suspicion of pulmonary embolism, the investigator should perfom exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigrapy.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be included in the long term safety follow up study (4 years).

6.2.1. Study assessments

- All AEs up to Day 28
- All AESI up to Year 1
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3, 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets
    - GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening
- Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)

- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28
- Chest x-ray,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)
- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion
- Transjugular liver biopsy: – only data collection in case the biopsy has been done during the same admission or previously – no transjugular liver biopsy is required for the study protocol.

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

<u>Thromboelastogram and thromboglobulin generation tests</u>

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min. twice). The plasma will be colleted and put in cryotubes (min. 2 tubes containing each min. 500 microL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Hippocrate 10,
1200 Woluwe-Saint Lambert BELGIUM

<u>Anti-HLA antibodies</u>

Serum will be collected and frozen.

Prof. Dominique Latinne

St Luc Hospital –Tour Franklin
Avenue Hippocrate 10 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

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Laboratory of Hepatology KU Leuven
Herestraat 49
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6.3. STUDY FLOW CHART

Table 6-1 Study Flowchart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	**Baseline**	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	**D21[b]**	**D28[c]**	**M2**	**M3**	**M6**	**M12**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

‡ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome
- Transplantation and outcome of the transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

Possibly

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probable

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definite

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

Moderate, when interfering with ADL;

Severe, when impeding with ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SAR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2.

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics.

Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences must "verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection".

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator's Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient's further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission

for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of Promethera members including medical monitor, pharmacovigilance representative, clinical representive and external members with expertise in liver disease or other relevant medical fields. The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.
2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.
    - When the first cohort 1 evaluable patient will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data.
    - When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advise on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.
    - When the first cohort 2 evaluable patient will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data.
5. The SMC will review severe thrombotic events assessed as related to HepaStem administration by the investigator.

6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- One or more biostatisticians
- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists may be invited to participate as non-voting members at any time if additional expertise is desired.

The frequency of SMC meetings will depend on several factors including the rate of enrolment, completion of five patients of the low dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL or renal replacement
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2	>300	≤300 - > 200	≤200
or SpO2/FiO2	>357	>214- ≤357	≤214

Arroyo et al. 2015

11.2. MELD SCORE

MELD Score based on
- serum Creatinin
- serum Bilirubin and
- INR

Chung et al. 2012

11.3. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 20 July 2016

Version Number: 1.2

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____

_____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 20 July 2016

Version Number: 1.2

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competente Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

_____ _____

Joëlle Thonnard, Director Clinical and Medical Affairs Date (dd/mm/yyyy)

_____ _____

Etienne Sokal, MD Chief Innovation & Scientific Officer Date (dd/mm/yyyy)

_____ _____

Silver Ocean Ventures SAS, CEO, represented by John Tchelingerian Date (dd/mm/yyyy)

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 1.3 – 26 Oct 2016

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Innovation & Scientific Officer John Tchelingerian, MD – Chief Executive Officer Joëlle Thonnard, MD- Clinical & Medical Affairs Director
MEDICAL MONITOR	Joëlle Thonnard, T.+32.471.563.423
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LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 1.3_26Oct2016
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Twelve (12) evaluable Patients
Number of Centers	5-10 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients. Study periods The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation. The study is divided in the following periods: screening period, active study period

	dived in treatment period and evaluation period, and long-term safety follow-up. Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members. • When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data. • When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advise on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized. • When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data.

	Furthermore, the 3 first patients of each cohort will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete sheme or premature stop).Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period. After completion of this study, patients will be invited to be followed-up in the long term safety follow up study (4 years).
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and thereafter for an additional period of 1 year in the long term safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at $50x10^6$ cells/ml equivalent to $250x10^6$ cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on patient's status. The infusion rate shall not exceed 1 ml per min. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 Billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion. In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 Billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before the first HepaStem infusion of the day.

<table>
<tr>
<td>Eligibility - Inclusion Criteria</td>
<td>
<u>Inclusion criteria</u>:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
<u>N.B:</u> In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan).

4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions

<u>ACLF grade 1 eligible subset:</u>

– liver failure plus cerebral and/or kidney dysfunction
– renal failure plus cerebral dysfunction
– cerebral failure plus kidney dysfunction
– coagulation failure plus cerebral and/or kidney dysfunction

<u>Or</u>

<u>ACLF grade 2 eligible subset</u>:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfuncytions or failures are defined according to CLIF-C OF score as below<u>:</u>

<u>Diagnostic criteria of kidney and cerebral dysfunction</u>

– kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL
– cerebral: moderate impairment of brain function as defined by grade I-II Hepatic Encephalopathy based on West Haven criteria

<u>Diagnostic criteria of organ failures</u>

– liver: serum bilirubin ≥ 12 mg/dL
– kidney: serum creatinine ≥ 2 mg/dL
– cerebral: grade III-IV HE based on West Haven criteria
– coagulation: international normalized ratio [INR] ≥ 2.5
</td>
</tr>
<tr>
<td>Eligibility - Exclusion Criteria</td>
<td>
<u>Exclusion criteria</u>:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.

2. Known prothrombotic disease or medical history of thrombotic events.
</td>
</tr>
</table>

	3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h. 4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 5. Clinical evidence of aspergilus infection. 6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria. 7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation. 8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion. 9. MELD score > 35. 10. Previous organ transplantation and/or ongoing immunosuppressive treatments. 11. Postoperative-decompensation following hepatectomy. 12. Renal failure due to chronic kidney disease. 13. Clinically significant left-right cardiac shunt. 14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions. 15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. 16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 18. Participation to any other interventional study within the last 4 weeks. 19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisouness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

	The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline.
	
	Secondary Endpoints:
	
	• Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
	  ○ Mortality
	  ○ Liver transplantation
	  ○ Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score.
	• Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
	  ○ Bilirubin, creatinine, INR and albumin values
	
	• Long term safety follow-up
	  ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1
	  ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of transplantation
	  ○ New ACLF episode will be summarized at Month 3 and Year 1

Study Assessment visits	Study visits During the screening and and treatment period, patients will be hospitalised. During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit). Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread

	over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given. on HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped. A study visit will be performed on Day 14 ± 2 days, including the evaluations listed below. On the other days during the hospital stay, patients will be followed-up according to usual practice. After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged. After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month). Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2). At the Month 12 study visit, patients will be invited to be included in the long term safety follow up study (4 years). <u>Study assessments</u> • All AEs up to Day 28 • All AESI up to Year 1 • Concomitant medication modifications up to Day 28 • Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12. • Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) : ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.

	○ Before, during and after infusion • West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3, 6 and 12 • Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12. ○ White Blood Cell count, Red Blood Cell count, platelets ○ GOT, GPT, bilirubin, alkaline phosphatase, γ GT, ○ Creatinine, Urea or BUN ○ CRP ○ INR, aPTT ○ Serum albumin, sodium, potassium, • Lipase: at screening • Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission) • Urine test (Sediment, Creat, Glc, Protein, Albm): at screening • Protein C, Protein S, anti-thrombin III: at screening • Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab) • Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab) • Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab) • Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 • Chest x-ray, • Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected) • ECG: at screening (if not performed during same admission) • Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion Transjugular liver biopsy: – only data collection in case the biopsy has been done during the same admission or previously – no transjugular liver biopsy is required for the study protocol.A SMC will review safety data and advice on study conduct. Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

	In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw. In case of liver transplantation, a sample of the explanted liver will be collected if possible.
Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics. Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who receive at least one infusion. All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by

	treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The Report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Study Flow chart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	**Baseline**	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	**D21[b]**	**D28[c]**	**M2**	**M3**	**M6**	**M12**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

‡ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

1. BACKGROUND AND RATIONALE

1.1. ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Epidemiological studies indicate that there is an increasing prevalence of liver cirrhosis related to chronic infection by hepatitis C or B virus, alcohol consumption and non-alcoholic steatohepatitis worldwide (Murray *et al.* 2012). The natural course of cirrhosis is from compensated to decompensated disease. Decompensation is characterized by the development of major complications of liver disease (variceal bleeding, ascites, hepatic encephalopathy and bacterial infections) and is associated with poor prognosis. In addition to acute decompensation, ACLF is characterized by organ/system failure(s) (liver, kidney, brain, coagulation, circulation and/or lung) and high short-term mortality (33% at 28 days and 51% at 90 days). Approximately 31% of patients admitted to hospital for acute decompensation of cirrhosis present ACLF at admission (20%) or develop the syndrome during hospitalization (11%) (Moreau *et al.* 2013). Mortality rate depends on the number of failing organs as defined by the CLIF-SOFA score or the CLIF-OF score (a simplified version of the CLIF-SOFA score) (Table 1-1) (Moreau et al. 2013, Arroyo et al. 2015). Three grades define ACLF severity (Table 1-2~~Table 1-2~~). ACLF grade 1, defined as single kidney failure or single "non-kidney" organ failure with serum creatinine of 1.5-1.9 mg/dL and/or hepatic encephalopathy grade 1-2, is the most prevalent form of ACLF (15.8% of patients admitted at hospital with acute decompensation) and has a 28-day mortality rate of 23%. Patients with ACLF grade 2 (2 failing organs; prevalence 10.9%) have an intermediate prognosis (28-day mortality rate of 31%). Finally, ACLF grade 3 (with 3 or more organ failures) is the less frequent form of ACLF (4.4%) but shows extremely high mortality rates reaching 75% at 28 days.

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years, frequently alcoholics, in relation to a systemic inflammatory reaction due to bacterial infections, acute alcoholic liver injury or, in 40% of patients, to as yet unidentified precipitating events (Moreau et al. 2013). The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion, which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 or renal replacement
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by

translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may causes necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidences support the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety

profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properites of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013). Later, the technology of large-scale cell production was transferred to Promethera Biosciences which produce clinical batches of HHALPC in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs). In immunodeficient (SCID) mice transplanted with liver-derived progenitor cells, evidence of presence of human hepatocyte-like cells in the liver supported the biological plausibility of cell engraftment (Najimi *et al.* 2007; Khuu *et al.* 2011; Khuu *et al.* 2013). Short-term biodistribution assessed in rats using cells labeled with oxine ^{111}-Indium showed that cells concentrated in the liver (until 72 hours) (Tondreau et al. in preparation). Risk of tumor formation has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. *In vitro* studies showed that liver-derived progenitor cells present a low immunogenic phenotype (Sana *et al.* 2014). These cells have a pro-coagulant effect, similar to bone marrow derived MSCs, which may favor thrombosis. A study showed that concomitant treatment with an antithrombin activator or direct factor Xa inhibitor and direct thrombin inhibitor proved to be a particularly effective combination for controlling the procoagulant effects both *in vitro* and *in vivo* (Stephenne *et al.* 2012) (Please refer to the IB for more details).

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

Selected patients under the hospital exemption regulation were treated with liver-derived progenitor cells infused *via* the portal vein. In one of them, short-term biodistribution assessed using cells labeled with oxine ^{111}-Indium showed liver biodistribution of the cells (Sokal *et al.* 2013; Defresne *et al.* 2014).

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg)

(4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and

morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. This data lays the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

A hospital exemption treatment was conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated well this cell infusion, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, right ankle, and spine, and had disappeared from the lungs.

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell

activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For examples, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012). In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

The doses reported in publications on MSCs evaluation in immune-mediated diseases are varying usually between 0.5 to 3 millions cells/kg/infusion (Vandermeulen et al. 2014, Sharma et al. 2014). Single infusion or repeated infusions are reported. For example, remestemcel-L (Prochymal®, Osiris Therapeutics) was given IV at a dose of 2 x 10^6 MSCs/kg of body weight twice weekly for 4 consecutive weeks. Patients received all 8 infusions in the initial treatment plan by day 28. Infusions were administered at least 3 days apart (Kurtzberg et al. 2014). In some reports, higher doses were given, up

to 800 millions cells/infusion (~11 millions/kg/infusion) (Ra et al. 2011, Mayer et al. 2013, Lublin et al. 2014, Melmed et al. 2015). In inflammatory liver diseases, doses varying between 0.5 to 5 millions cells have been reported (Berardis et al. 2015). To be noted, the mode of administration was IV route and no concomitant administration of anti-coagulation medication was reported.

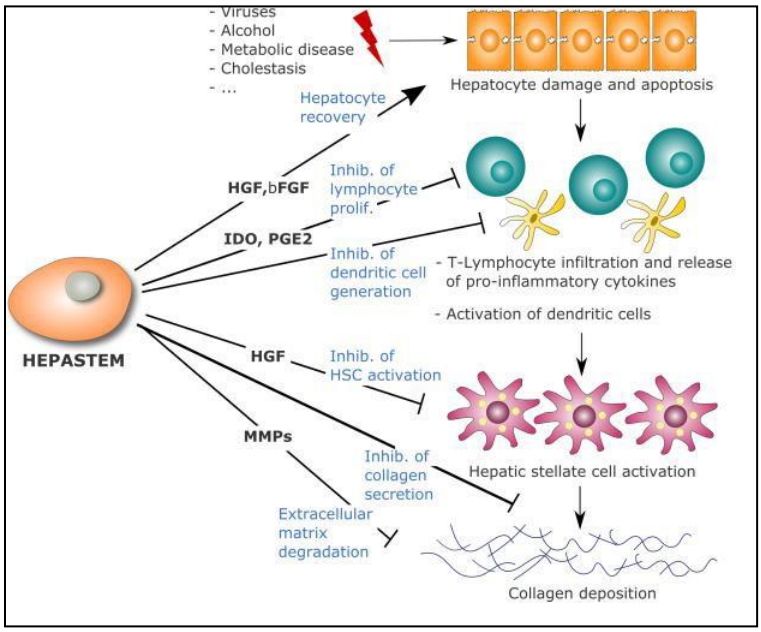
1.2.6. Pre-clinical immunomodulatory data of liver-derived progenitor cells

The first transcriptomics and secretomics tests performed on liver-derived progenitor cells grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models present important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.7. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing in the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.8. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts of 6 ACLF patients each. The study will include patients with ACLF grade 1 or 2 (excluding those with renal organ failure only, or those with circulatory or respiratory failure). It is planned to have a first group of 6 patients (cohort 1) being administered with 4 infusions of 250 millions cells each. Once this has been proven safe, a second group of 6 patients (cohort 2) will receive 4 infusions of 500 milions cells each. The infusions will be administered over 2 weeks with the first infusion started within a few days after patient's hospitalisation due to acute liver decompensation leading to ACLF. HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period.

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 6 patients, appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

HepaStem administration will be started rapidly after hospitalisation and will be completed within 2 weeks. This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of ACLF patients. As ACLF is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up for safety up 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining design of future efficacy clinical studies.

Study population

The selected adult population presents an expected mortality rate at short-term (28 days) close to 30%, which justify the use of a novel approach carrying potential short term benefits based on its immunomodulatory and anti-inflammatory properties. The short term mortality risk at 28 days is estimated close to 23% for ACLF grade 1 or 31% for ACLF grade 2. Patients with ACLF grade 1 with kidney

failure only will be excluded as their mortality risk at 28 days is actually close (< 20%) to that of patients with one (non-kidney) organ failure only (No ACLF) (Moreau et al. 2013). Patients with ACLF grade 3 will be excluded as they have a mortality rate reaching 75% at 28 days, making difficult to assess any safety or efficacy cell effect in this group. ACLF mainly occurs in cirrhotic patients aged around 50-60 years.

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 14 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 28 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 millions cells per infusion) is close to MSC doses given in other trials for immune mediated inflammatory diseases (Section 1.2.5), therefore, it is expected to show similar safety and efficacy profile. It corresponds also to the dose of liver-dirived progenitor cells administered via IV to the heamophila patient (see 1.2.4). The second selected dose represents a two-fold increase, still in the range of doses reported for MSCs. In addition, both doses are in the low range compared to HepaStem doses given in HEP001 paediatric study where administration of 500 millions cells per day was shown to be safe and well tolerated (see 1.2.3).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 UI/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damages nor any respiratory symptoms, before homing mainly in the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in ACLF patients, especially since it allows repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs have also a

procoagulant activity comparable to liver-derived progenitor cells (Stephenne et al. 2012), nevertheless literature report show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015). Furthermore, it has been established that hemostatic potential in patients with chronic liver disease is in a rebalanced status due to concomittant decrease in pro and anti-hemostatic drivers. In ACLF patients, the inflammatory process may trigger the unstable balance of hemostasis of cirrhotic patients to any of two states and may be manifested by either bleeding or thrombotic complications (Blasco-Algora et al. 2015). Thus an anti-coagulation may be contra-indicated in ACLF patients as they could be at risk of gastrointestinal hemorrhage, risks that may not be assessed by coagulation tests (prothombine time, INR, thrombin generation and thromboelastometry) (Lisman et al. 2012, Stravitz et al. 2012, Tripodi et al. 2009a, Tripodi et al. 2009b). Furthermore, bivalirudin use is not validated in cirrhotic patients. In order to mitigate risks of thrombosis in ACLF patients receiving HepaStem, several precautionary measures will be taken, as described in Section 5.5.1.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

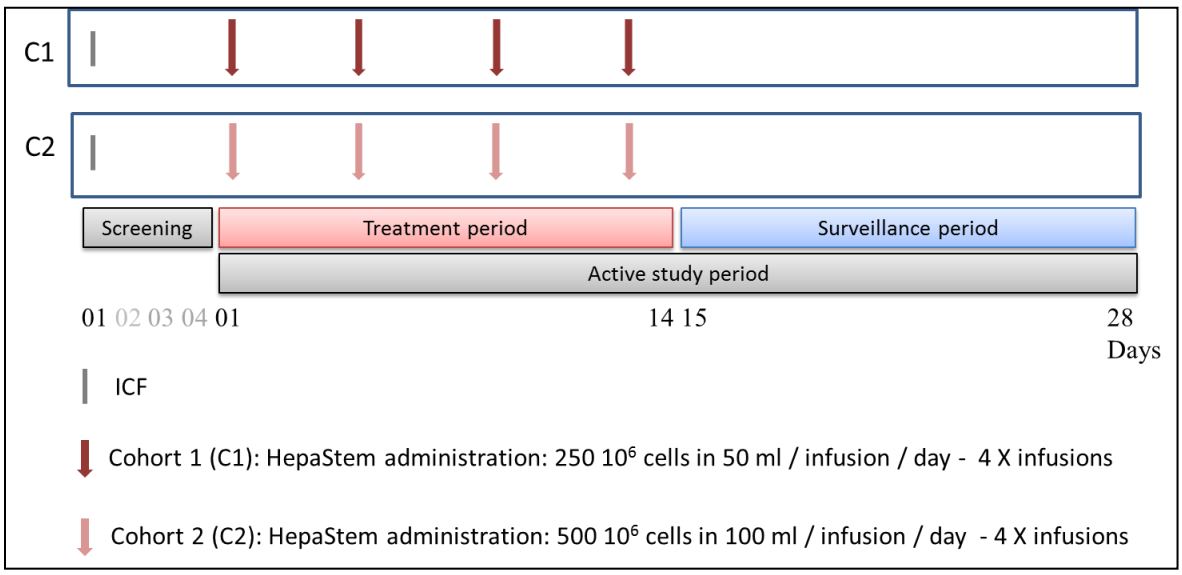
Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



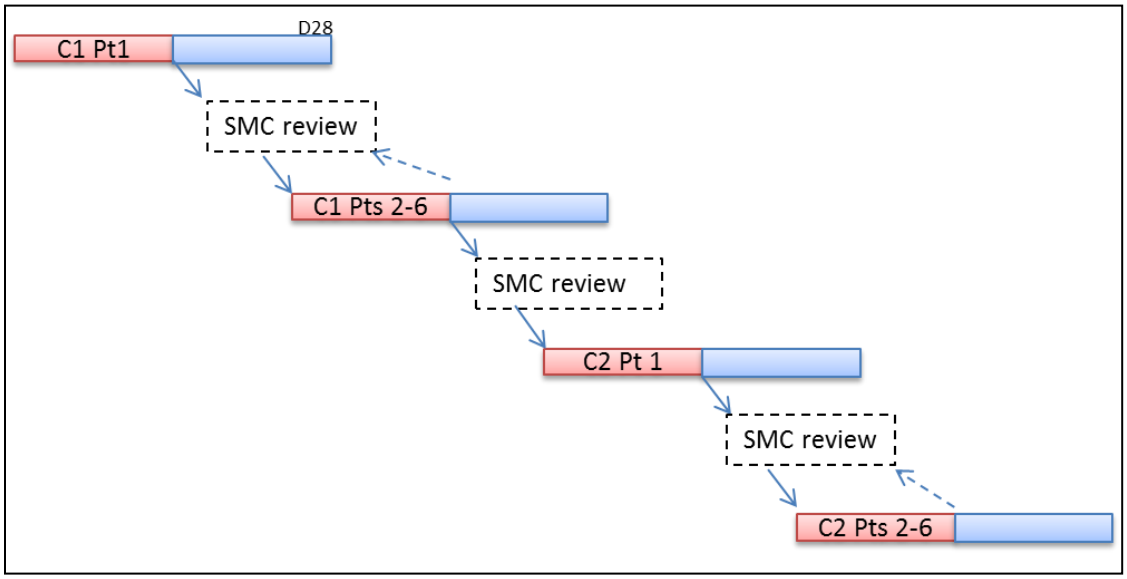
The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members (See Section 9.13):

- When the first cohort 1 evaluable patient will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

- When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

- When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring Committe



The study assessments are described in Section 6.

Furthermore, the 3 first patients of each cohort will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete scheme or premature stop). The sequential approach will be under the control of Promethera (based on review of eligibility criteria by the medical monitor and HepaStem delivery). In case of safety signal, the SMC will be involved in the AEs review and evaluation, and the SMC will advise on further inclusion.

These measures (SMC meetings and sequential treatment for the 3 first patients in each cohort) will allow respecting the progress of dose levels with limited risk for the patients.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be invited to be followed-up in the long term safety follow up study (4 years).

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
  - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of transplantation
- New ACLF episode will be summarized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in 2 successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in 5 to 10 clinical centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. The hospitalisation unit will be adapted according to the medical status of the patient and the organization of study center hospital. Patients with a low CLIF-OF score will be more likely included in the hepatology department (standard or intermediate care unit), while the patient with high CLIF-OF score will more likely be included in the Intensive Care Unit.

Patients will remain hospitalised at least during the treatment period. During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Patients with ACLF at first evaluation post-admission or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
   N.B.: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

   - liver histology or
   - clinical and imaging examination (may include fibroscan).

4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions

ACLF grade 1 eligible subset:

   – liver **failure** plus cerebral and/or kidney **dysfunction**

- renal **failure** plus cerebral **dysfunction**
- cerebral **failure** plus kidney **dysfunction**
- coagulation **failure** plus cerebral and/or kidney **dysfunction**

Or

ACLF grade 2 eligible subset:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfuncytions or failures are defined according to CLIF-C OF score as below

Diagnostic criteria of kidney and cerebral dysfunction

- kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL.
- cerebral: moderate impairment of brain function as defined by grade I-II HE based on West Haven criteria.

Diagnostic criteria of organ failures

- liver: serum bilirubin ≥ 12 mg/dL;
- kidney: serum creatinine ≥ 2 mg/dL;
- cerebral: grade III-IV HE based on West Haven criteria;
- coagulation: international normalized ratio [INR] ≥ 2.5

For both grades, patients with circulatory and/or respiratory failure are excluded (see exclusion criteria 6 and 7).

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Known prothrombotic disease or medical history of thrombotic events.
3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
5. Clinical evidence of aspergilus infection.
6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria.

7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion.
9. MELD score > 35.
10. Previous organ transplantation and/or ongoing immunosuppressive treatments.
11. Postoperative-decompensation following hepatectomy.
12. Renal failure due to chronic kidney disease.
13. Clinically significant left-right cardiac shunt.
14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
18. Participation to any other interventional study within the last 4 weeks.
19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration for a patient are the following:

Transitory discontinuation:

- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem. Infusions may be restarted after recovery. If planned infusions are not performed within the 2 week treatment period (± 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, *i.e.*, thrombosis, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock, severe worsening of hepatic function.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAW CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No Patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. ACLF resolution or detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1~~Table 5-1~~).

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Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2~~Table 5-2~~).

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Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and

the syringe will be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days.

In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes.

In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate or ICUs or standard units, depending on the medical status of the patient and the organisation of study center hospital. Regardless of the unit of hospitalization, patients will remain hospitalised at least during the treatment period, with a close monitoring of each patient. During HepaStem infusion, a continuous monitoring of the vital signs of the patient is required.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension have to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics and risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis, respiratory disorder as cells first transit to the lungs, hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the below mentioned risks, there might be other, at this time, unknown risks.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lungs disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect. **Number of cells administered per infusion will be maximum 500 millions cells and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min**. The lower dose regimen will be applied before the higher one. These doses are in the low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication (See Section 1).

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions. Any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with medical history of thrombotic events or known prothrombotic disease will be excluded from the study.

In case of clinical signs or suspicion of pulmonary embolism, the investigator should perform exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigraphy.

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Long Term Safety follow-up Period of 1 year. Thereafter, patients will be invited to be followed-up in a long term safety follow up study (4 years).

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and treatment period, patients will be hospitalised in intermediate or ICUs or standard units, depending of the severity of the patient disease.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period ($\pm$ 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given.

On HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

During the infusion, the patient will be continuously monitored for early detection of any potential AEs.

A study visit will be performed on Day 14 $\pm$ 2 days, including the evaluations listed below.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

In case of any suspicion of AE, the investigator will perform the exams she/he evaluates as appropriate. In particular, any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement. In case of clinical signs or suspicion of pulmonary embolism, the investigator should perfom exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigrapy.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be included in the long term safety follow up study (4 years).

6.2.1. Study assessments

- All AEs up to Day 28
- All AESI up to Year 1
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3, 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets
    - GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening

- Protein C, Protein S, anti-thrombin III: at screening
- Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28
- Chest x-ray,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)
- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion
- Transjugular liver biopsy: – only data collection in case the biopsy has been done during the same admission or previously – no transjugular liver biopsy is required for the study protocol.

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

<u>Thromboelastogram and thromboglobulin generation tests</u>

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min. twice). The plasma will be colleted and put in cryotubes (min. 2 tubes containing each min. 500 microL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Hippocrate 10,
1200 Woluwe-Saint Lambert BELGIUM

Anti-HLA antibodies

Serum will be collected and frozen.

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Hippocrate 10 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHART

Table 6-1 Study Flowchart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	**Baseline**	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	**D21[b]**	**D28[c]**	**M2**	**M3**	**M6**	**M12**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

‡ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome
- Transplantation and outcome of the transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section ~~7.2.3~~7.2.3
- Assessment of causal relationship to study treatment, as in Section ~~7.2.2~~7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

Unrelated

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

Unlikely

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

Possibly

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probable

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definite

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

Moderate, when interfering with ADL;

Severe, when impeding with ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SAR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2.

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics.

Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences must "verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection".

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator's Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient's further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission

for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of Promethera members including medical monitor, pharmacovigilance representative, clinical representive and external members with expertise in liver disease or other relevant medical fields. The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.
2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.
    - When the first cohort 1 evaluable patient will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data.
    - When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advise on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.
    - When the first cohort 2 evaluable patient will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data.
5. The SMC will review severe thrombotic events assessed as related to HepaStem administration by the investigator.

6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- One or more biostatisticians
- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists may be invited to participate as non-voting members at any time if additional expertise is desired.

The frequency of SMC meetings will depend on several factors including the rate of enrolment, completion of five patients of the low dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL or renal replacement
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2	>300	≤300 - > 200	≤200
or SpO2/FiO2	>357	>214- ≤357	≤214

Arroyo et al. 2015

11.2. MELD SCORE

MELD Score based on
- serum Creatinin
- serum Bilirubin and
- INR

Chung et al. 2012

11.3. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 20 July 2016

Version Number: 1.2

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____

_____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 20 July 2016

Version Number: 1.2

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competente Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

_____ _____

Joëlle Thonnard, Director Clinical and Medical Affairs Date (dd/mm/yyyy)

_____ _____

Etienne Sokal, MD Chief Innovation & Scientific Officer Date (dd/mm/yyyy)

_____ _____

Silver Ocean Ventures SAS, CEO, represented by John Tchelingerian Date (dd/mm/yyyy)

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 2.0 – 13 Dec 2016

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Innovation & Scientific Officer John Tchelingerian, MD – Chief Executive Officer Joëlle Thonnard, MD- Clinical & Medical Affairs Director Abdel Ladouzi, Pharm D - Clinical Operations Director
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LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 2.0 - 13 Dec 2016
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Twelve (12) evaluable Patients
Number of Centers	5-10 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients. Study periods The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation.

	The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up. Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members. • When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data. • When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient. • When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

	Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period. After completion of this study, patients will be followed-up in the Product registry.
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50x106 cells/ml equivalent to 250x106 cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on patient's status. The infusion rate shall not exceed 1 ml per min. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 Billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion. In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 Billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before the first HepaStem infusion of the day.
Eligibility - Inclusion Criteria	Inclusion criteria: 1. Adult aged between 18 and 70 year old. 2. Informed Consent.

<table>
<tr><td></td><td>
N.B: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan).

4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions

ACLF grade 1 eligible subset:

– liver failure plus cerebral and/or kidney dysfunction
– renal failure plus cerebral dysfunction
– cerebral failure plus kidney dysfunction
– coagulation failure plus cerebral and/or kidney dysfunction

Or

ACLF grade 2 eligible subset:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfuncytions or failures are defined according to CLIF-C OF score as below:

Diagnostic criteria of kidney and cerebral dysfunction

– kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL
– cerebral: moderate impairment of brain function as defined by grade I-II Hepatic Encephalopathy based on West Haven criteria

Diagnostic criteria of organ failures

– liver: serum bilirubin ≥ 12 mg/dL
– kidney: serum creatinine ≥ 2 mg/dL or serum creatinine < 2 mg/dL because corrected by Terlipressine should be considered as an organ failure
– cerebral: grade III-IV HE based on West Haven criteria
– coagulation: international normalized ratio [INR] ≥ 2.5
</td></tr>
<tr><td>Eligibility - Exclusion Criteria</td><td>
Exclusion criteria:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.

2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.
</td></tr>
</table>

	Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion. 3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h. 4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 5. Clinical evidence of aspergilus infection. 6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria. 7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation. 8. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period. 9. MELD score > 35. 10. Previous organ transplantation and/or ongoing immunosuppressive treatments. 11. Postoperative-decompensation following hepatectomy. 12. Renal failure due to chronic kidney disease. 13. Clinically significant left-right cardiac shunt. 14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions. 15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. 16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 18. Participation to any other interventional study within the last 4 weeks. 19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisouness, severity, relationship to IMP and/or IMP administration procedure

	The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.
	
	The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline.
	
	<u>Secondary Endpoints</u>:
	
	• Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
	  ○ Mortality
	  ○ Liver transplantation
	  ○ Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score.
	• Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
	  ○ Bilirubin, creatinine, INR and albumin values
	
	• Long term safety follow-up
	  ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1
	  ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation
	  ○ New ACLF episode will be summarized at Month 3 and Year 1

Study Assessment visits	<u>Study visits</u> During the screening and and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given.

	on HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.
	
	A study visit will be performed on Day 14 ± 2 days, including the evaluations listed below.
	
	On the other days during the hospital stay, patients will be followed-up according to usual practice.
	
	After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.
	
	After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).
	
	Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).
	
	At the Month 12 study visit, patients will be invited to be included in the Product Registry.
	
	<u>Study assessments</u>
	
	• All AEs up to Day 28
	• All AESI up to Year 1
	• Concomitant medication modifications up to Day 28
	• Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3. 6 and 12

	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ White Blood Cell count, Red Blood Cell count, platelets
	  ○ GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
	  ○ Creatinine, Urea or BUN
	  ○ CRP
	  ○ INR, aPTT
	  ○ Serum albumin, sodium, potassium,
	• Lipase: at screening
	• Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
	• Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
	• Protein C, Protein S, anti-thrombin III: at screening
	• Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab)
	• Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
	• Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab)
	• Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28
	• Chest x-ray,
	• Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
	• ECG: at screening (if not performed during same admission)
	• Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion
	• A transjugular liver biopsy: optional (if not already performed during the same admission)
	
	A SMC will review safety data and advice on study conduct.
	
	Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.
	
	In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

	In case of liver transplantation, a sample of the explanted liver will be collected if possible.
Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics. Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who receive at least one infusion. All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

	All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The Report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Study Flow chart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	**Baseline**	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	**D21[b]**	**D28[c]**	**M2**	**M3**	**M6**	**M12**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

ǂ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

1. BACKGROUND AND RATIONALE

1.1. ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Epidemiological studies indicate that there is an increasing prevalence of liver cirrhosis related to chronic infection by hepatitis C or B virus, alcohol consumption and non-alcoholic steatohepatitis worldwide (Murray *et al.* 2012). The natural course of cirrhosis is from compensated to decompensated disease. Decompensation is characterized by the development of major complications of liver disease (variceal bleeding, ascites, hepatic encephalopathy and bacterial infections) and is associated with poor prognosis. In addition to acute decompensation, ACLF is characterized by organ/system failure(s) (liver, kidney, brain, coagulation, circulation and/or lung) and high short-term mortality (33% at 28 days and 51% at 90 days). Approximately 31% of patients admitted to hospital for acute decompensation of cirrhosis present ACLF at admission (20%) or develop the syndrome during hospitalization (11%) (Moreau *et al.* 2013). Mortality rate depends on the number of failing organs as defined by the CLIF-SOFA score or the CLIF-OF score (a simplified version of the CLIF-SOFA score) (Table 1-1) (Moreau et al. 2013, Arroyo et al. 2015). Three grades define ACLF severity (Table 1-2). ACLF grade 1, defined as single kidney failure or single "non-kidney" organ failure with serum creatinine of 1.5-1.9 mg/dL and/or hepatic encephalopathy grade 1-2, is the most prevalent form of ACLF (15.8% of patients admitted at hospital with acute decompensation) and has a 28-day mortality rate of 23%. Patients with ACLF grade 2 (2 failing organs; prevalence 10.9%) have an intermediate prognosis (28-day mortality rate of 31%). Finally, ACLF grade 3 (with 3 or more organ failures) is the less frequent form of ACLF (4.4%) but shows extremely high mortality rates reaching 75% at 28 days.

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years, frequently alcoholics, in relation to a systemic inflammatory reaction due to bacterial infections, acute alcoholic liver injury or, in 40% of patients, to as yet unidentified precipitating events (Moreau et al. 2013). The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion, which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 or renal replacement
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by translocation of

bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may causes necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidences support the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory

disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properites of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013). Later, the technology of large-scale cell production was transferred to Promethera Biosciences which produce clinical batches of HHALPC in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs). In immunodeficient (SCID) mice transplanted with liver-derived progenitor cells, evidence of presence of human hepatocyte-like cells in the liver supported the biological plausibility of cell engraftment (Najimi *et al.* 2007; Khuu *et al.* 2011; Khuu *et al.* 2013). Short-term biodistribution assessed in rats using cells labeled with oxine ^{111}-Indium showed that cells concentrated in the liver (until 72 hours) (Tondreau et al. in preparation). Risk of tumor formation has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. *In vitro* studies showed that liver-derived progenitor cells present a low immunogenic phenotype (Sana *et al.* 2014). These cells have a pro-coagulant effect, similar to bone marrow derived MSCs, which may favor thrombosis. A study showed that concomitant treatment with an antithrombin activator or direct factor Xa inhibitor and direct thrombin inhibitor proved to be a particularly effective combination for controlling the procoagulant effects both *in vitro* and *in vivo* (Stephenne *et al.* 2012) (Please refer to the IB for more details).

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

Selected patients under the hospital exemption regulation were treated with liver-derived progenitor cells infused *via* the portal vein. In one of them, short-term biodistribution assessed using cells labeled with oxine ^{111}-Indium showed liver biodistribution of the cells (Sokal *et al.* 2013; Defresne *et al.* 2014).

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg) (4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in

cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. This data lays the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

A hospital exemption treatment was conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated well this cell infusion, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, right ankle, and spine, and had disappeared from the lungs.

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor

(HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For examples, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012). In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

The doses reported in publications on MSCs evaluation in immune-mediated diseases are varying usually between 0.5 to 3 millions cells/kg/infusion (Vandermeulen et al. 2014, Sharma et al. 2014). Single infusion or repeated infusions are reported. For example, remestemcel-L (Prochymal®, Osiris Therapeutics) was given IV at a dose of 2 x 10^6 MSCs/kg of body weight twice weekly for 4 consecutive weeks. Patients received all 8 infusions in the initial treatment plan by day 28. Infusions were administered at least 3 days apart (Kurtzberg et al. 2014). In some reports, higher doses were given, up to 800 millions cells/infusion (~11 millions/kg/infusion) (Ra et al. 2011, Mayer et al. 2013, Lublin et al. 2014, Melmed et al. 2015). In inflammatory liver diseases, doses varying between 0.5 to 5 millions cells have been reported (Berardis et

al. 2015). To be noted, the mode of administration was IV route and no concomitant administration of anti-coagulation medication was reported.

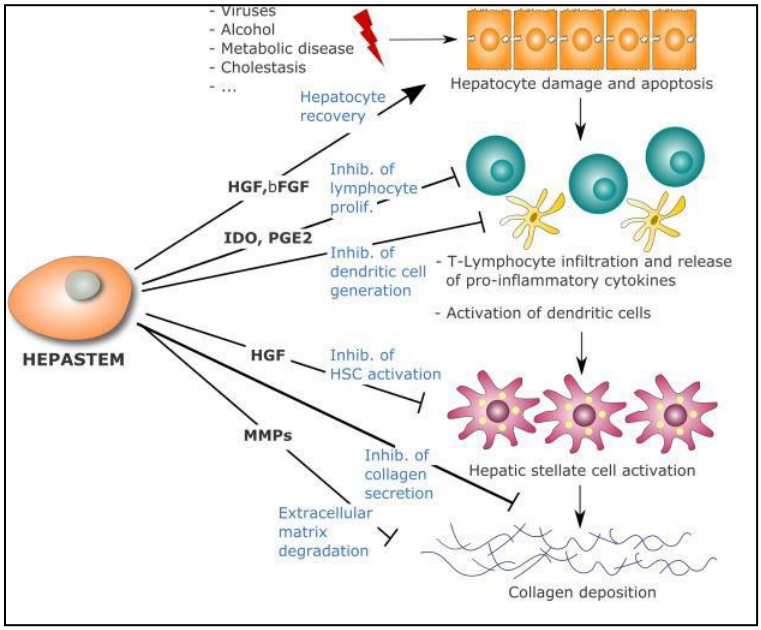
1.2.6. Pre-clinical immunomodulatory data of liver-derived progenitor cells

The first transcriptomics and secretomics tests performed on liver-derived progenitor cells grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models present important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.7. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing in the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.8. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts of 6 ACLF patients each. The study will include patients with ACLF grade 1 or 2 (excluding those with renal organ failure only, or those with circulatory or respiratory failure). It is planned to have a first group of 6 patients (cohort 1) being administered with 4 infusions of 250 millions cells each. Once this has been proven safe, a second group of 6 patients (cohort 2) will receive 4 infusions of 500 milions cells each. The infusions will be administered over 2 weeks with the first infusion started within a few days after patient's hospitalisation due to acute liver decompensation leading to ACLF. HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period.

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 6 patients, appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

HepaStem administration will be started rapidly after hospitalisation and will be completed within 2 weeks. This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of ACLF patients. As ACLF is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up for safety up 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining design of future efficacy clinical studies.

Study population

The selected adult population presents an expected mortality rate at short-term (28 days) close to 30%, which justify the use of a novel approach carrying potential short term benefits based on its immunomodulatory and anti-inflammatory properties. The short term mortality risk at 28 days is estimated close to 23% for ACLF grade 1 or 31% for ACLF grade 2. Patients with ACLF grade 1 with kidney failure only will be excluded as their mortality risk at 28 days is actually close (< 20%) to that of patients

with one (non-kidney) organ failure only (No ACLF) (Moreau et al. 2013). Patients with ACLF grade 3 will be excluded as they have a mortality rate reaching 75% at 28 days, making difficult to assess any safety or efficacy cell effect in this group. ACLF mainly occurs in cirrhotic patients aged around 50-60 years.

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 21 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 42 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 millions cells per infusion) is close to MSC doses given in other trials for immune mediated inflammatory diseases (Section 1.2.5), therefore, it is expected to show similar safety and efficacy profile. It corresponds also to the dose of liver-dirived progenitor cells administered via IV to the heamophila patient (see 1.2.4). The second selected dose represents a two-fold increase, still in the range of doses reported for MSCs. In addition, both doses are in the low range compared to HepaStem doses given in HEP001 paediatric study where administration of 500 millions cells per day was shown to be safe and well tolerated (see 1.2.3).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 UI/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damages nor any respiratory symptoms, before homing mainly in the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in ACLF patients, especially since it allows repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs have also a procoagulant activity comparable to liver-derived progenitor cells (Stephenne et al. 2012), nevertheless literature report

show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015). Furthermore, it has been established that hemostatic potential in patients with chronic liver disease is in a rebalanced status due to concomittant decrease in pro and anti-hemostatic drivers. In ACLF patients, the inflammatory process may trigger the unstable balance of hemostasis of cirrhotic patients to any of two states and may be manifested by either bleeding or thrombotic complications (Blasco-Algora et al. 2015). Thus an anti-coagulation may be contra-indicated in ACLF patients as they could be at risk of gastrointestinal hemorrhage, risks that may not be assessed by coagulation tests (prothombine time, INR, thrombin generation and thromboelastometry) (Lisman et al. 2012, Stravitz et al. 2012, Tripodi et al. 2009a, Tripodi et al. 2009b). Furthermore, bivalirudin use is not validated in cirrhotic patients. In order to mitigate risks of thrombosis in ACLF patients receiving HepaStem, several precautionary measures will be taken, as described in Section 5.5.1.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

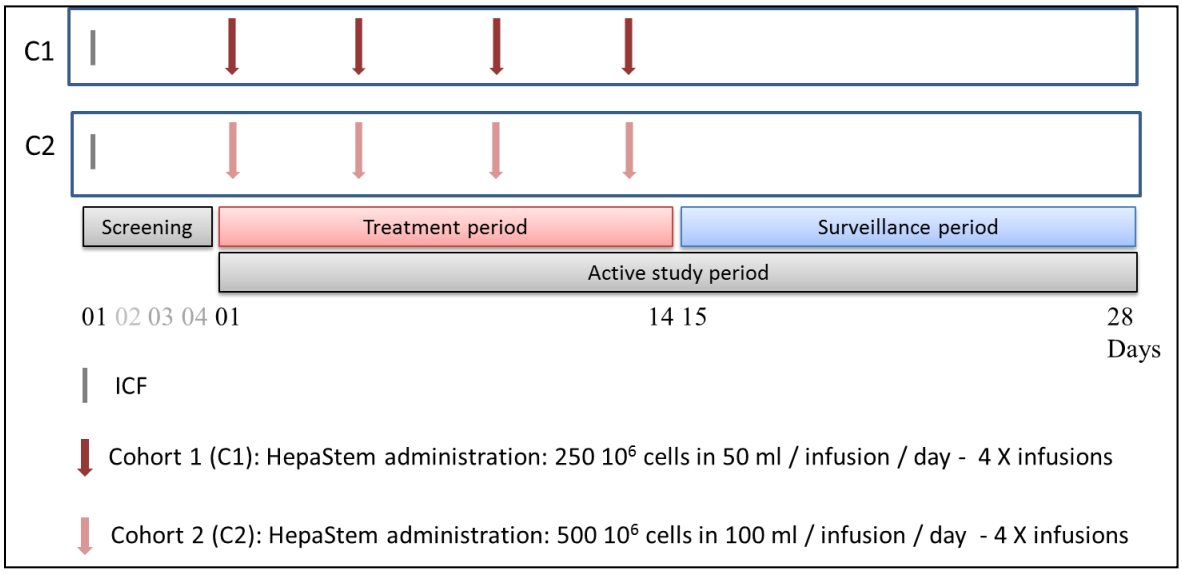
Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

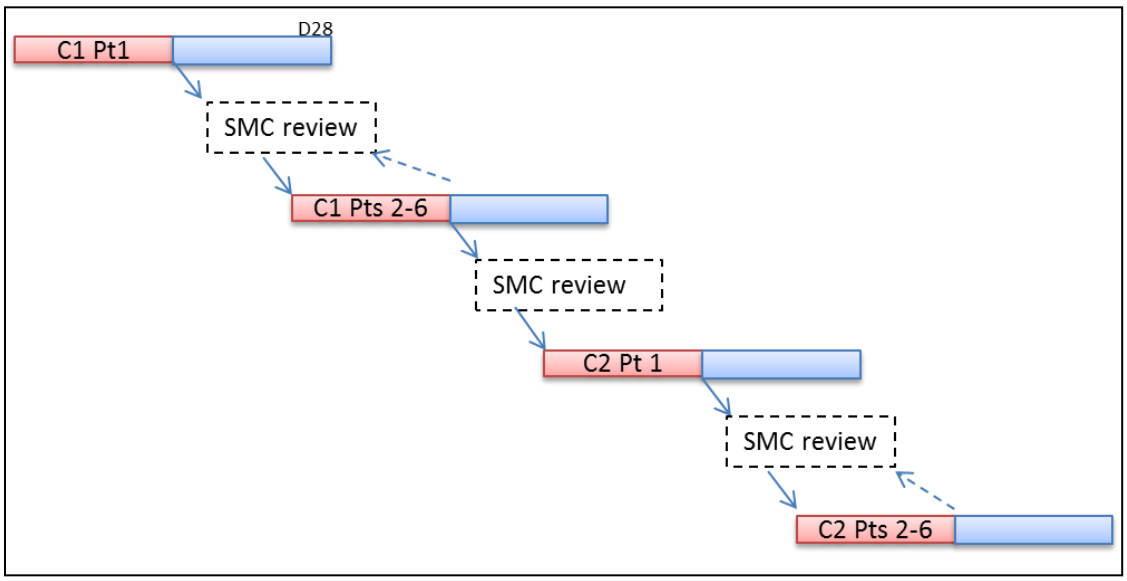
Figure 3-1 Study scheme of active study period



The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members (See Section 9.13):

- When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.
- When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient.
- When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring Committe



The study assessments are described in Section 6.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be followed-up in the Product registry.

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
  - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation
- New ACLF episode will be sumamrized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in 2 successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in 5 to 10 clinical centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days ($\pm$ 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Patients with ACLF at first evaluation post-admission or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
   N.B: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

   - liver histology or
   - clinical and imaging examination (may include fibroscan).

4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions

ACLF grade 1 eligible subset:

- liver **failure** plus cerebral and/or kidney **dysfunction**
- renal **failure** plus cerebral **dysfunction**
- cerebral **failure** plus kidney **dysfunction**
- coagulation **failure** plus cerebral and/or kidney **dysfunction**

Or

ACLF grade 2 eligible subset:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfuncytions or failures are defined according to CLIF-C OF score as below

Diagnostic criteria of kidney and cerebral dysfunction

- kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL.
- cerebral: moderate impairment of brain function as defined by grade I-II HE based on West Haven criteria.

Diagnostic criteria of organ failures

- liver: serum bilirubin ≥ 12 mg/dL;
- kidney: serum creatinine ≥ 2 mg/dL or serum creatinine < 2 mg/dL because corrected by Terlipressine should be considered as an organ failure;
- cerebral: grade III-IV HE based on West Haven criteria;
- coagulation: international normalized ratio [INR] ≥ 2.5

For both grades, patients with circulatory and/or respiratory failure are excluded (see exclusion criteria 6 and 7).

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.

   Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.
3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
5. Clinical evidence of aspergilus infection.
6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria.
7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
8. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.
9. MELD score > 35.
10. Previous organ transplantation and/or ongoing immunosuppressive treatments.

11. Postoperative-decompensation following hepatectomy.
12. Renal failure due to chronic kidney disease.
13. Clinically significant left-right cardiac shunt.
14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
18. Participation to any other interventional study within the last 4 weeks.
19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration are the following:

Transitory discontinuation:

- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem. Infusions may be restarted after recovery. If planned infusions are not performed within the 2 week treatment period (± 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAW CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No Patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. ACLF resolution or detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be

reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and the syringe will be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days.

In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes.

In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate or ICUs or standard units. Patients will be hospitalised during HepaStem treatment period.

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension have to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics and risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis, respiratory disorder as cells first transit to the lungs, hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the below mentioned risks, there might be other, at this time, unknown risks.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lungs disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect. **Number of cells administered per infusion will be maximum 500 millions cells and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min**. The lower dose regimen will be applied before the higher one. These doses are in the low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication (See Section 1).

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions.

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion will be excluded from the study.

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity

was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Long Term Safety follow-up Period of 1 year. Thereafter, they will be followed-up the the product registry.

Thereafter, patients will be followed in the Product registry.

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given.

on HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

A study visit will be performed on Day 14 ± 2 days, including the evaluations listed below.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be included in the Product Registry.

6.2.1. Study assessments

- All AEs up to Day 28
- All AESI up to Year 1
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets
    - GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening
- Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28
- Chest x-ray,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)

- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion
- A transjugular liver biopsy: optional (if not already performed during the same admission)

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

Thromboelastogram and thromboglobulin generation tests

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min. twice). The plasma will be colleted and put in cryotubes (min. 2 tubes containing each min. 500 microL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Hippocrate 10,
1200 Woluwe-Saint Lambert BELGIUM

Anti-HLA antibodies

Serum will be collected and frozen.

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Hippocrate 10 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHARTS

Table 6-1 Study Flowchart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	Baseline	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	D21[b]	D28[c]	M2	M3	M6	M12
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

‡ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome
- Liver Transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

Possibly

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probable

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definite

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

Moderate, when interfering with ADL;

Severe, when impeding with ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SAR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2.

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics.

Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated.

Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable

privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences must "verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection".

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator's Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient's further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the “Protocol Deviation Form” (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences’ monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients,

safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of Promethera members including medical monitor, pharmacovigilance representative, clinical representive and external members with expertise

in liver disease or other relevant medical fields. The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.
2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.
    - When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.
    - When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient.
    - When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.
5. The SMC will review severe thrombotic events assessed as related to HepaStem administration by the investigator.
6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- One or more biostatisticians
- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists may be invited to participate as non-voting members at any time if additional expertise is desired.

The frequency of SMC meetings will depend on several factors including the rate of enrollment, completion of five patients of the low dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL or renal replacement
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	 >300 >357	 ≤300 - > 200 >214- ≤357	 ≤200 ≤214

Arroyo et al. 2015

11.2. MELD SCORE

MELD Score based on
- serum Creatinin
- serum Bilirubin and
- INR

Chung et al. 2012

11.3. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 13 Dec 2016

Version Number: 2.0

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____ _____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 13 Dec 2016

Version Number: 2.0

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competente Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

_____ _____

Joëlle Thonnard, Director Clinical and Medical Affairs Date (dd/mm/yyyy)

_____ _____

Etienne Sokal, MD Chief Innovation & Scientific Officer Date (dd/mm/yyyy)

_____ _____

John Tchelingerian, CEO Date (dd/mm/yyyy)

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 2.1 – 20 Dec 2016

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Innovation & Scientific Officer John Tchelingerian, MD – Chief Executive Officer Joëlle Thonnard, MD- Clinical & Medical Affairs Director
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MONITORING & STUDY COORDINATION	Virginie Barthel, T.+32.471.784.097
SAFETY & PHARMACOVIGILANCE	pv@promethera.com **T.*+32.10.39.43.43***

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LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 2.1_20Dec2016
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Twelve (12) evaluable Patients
Number of Centers	5-10 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients. Study periods The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation. The study is divided in the following periods: screening period, active study period

	dived in treatment period and evaluation period, and long-term safety follow-up. Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members. • When the first cohort 1 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data. • When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advise on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized. • When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data.

	Furthermore, the 3 first patients of each cohort will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete sheme or premature stop).Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period. After completion of this study, patients will be invited to be followed-up in the long term safety follow up study (4 years).
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and thereafter for an additional period of 1 year in the long term safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at $50x10^6$ cells/ml equivalent to $250x10^6$ cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on patient's status. The infusion rate shall not exceed 1 ml per min. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 Billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion. In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 Billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before the first HepaStem infusion of the day.

<table>
<tr>
<td>Eligibility - Inclusion Criteria</td>
<td>
<u>Inclusion criteria</u>:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
<u>N.B:</u> In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan).

4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions

<u>ACLF grade 1 eligible subset:</u>

– liver failure plus cerebral and/or kidney dysfunction
– renal failure plus cerebral dysfunction
– cerebral failure plus kidney dysfunction
– coagulation failure plus cerebral and/or kidney dysfunction

<u>Or</u>

<u>ACLF grade 2 eligible subset</u>:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfuncytions or failures are defined according to CLIF-C OF score as below<u>:</u>

<u>Diagnostic criteria of kidney and cerebral dysfunction</u>

– kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL
– cerebral: moderate impairment of brain function as defined by grade I-II Hepatic Encephalopathy based on West Haven criteria

<u>Diagnostic criteria of organ failures</u>

– liver: serum bilirubin ≥ 12 mg/dL
– kidney: serum creatinine ≥ 2 mg/dL or serum creatinine < 2 mg/dL becau
Terlipressine should be considered as an organ failure
– cerebral: grade III-IV HE based on West Haven criteria
– coagulation: international normalized ratio [INR] ≥ 2.5
</td>
</tr>
<tr>
<td>Eligibility - Exclusion Criteria</td>
<td>
<u>Exclusion criteria</u>:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
</td>
</tr>
</table>

	2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion. 3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h. 4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 5. Clinical evidence of aspergilus infection. 6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria. 7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation. 8. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period 9. MELD score > 35. 10. Previous organ transplantation and/or ongoing immunosuppressive treatments. 11. Postoperative-decompensation following hepatectomy. 12. Renal failure due to chronic kidney disease. 13. Clinically significant left-right cardiac shunt. 14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions. 15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. 16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 18. Participation to any other interventional study within the last 4 weeks. 19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	Primary endpoint: Safety

	• AEs reported up to Day 28 of the active study period, assessed for serisouness, severity, relationship to IMP and/or IMP administration procedure
	
	The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.
	
	The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline.
	
	<u>Secondary Endpoints</u>:
	
	• Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
	  ○ Mortality
	  ○ Liver transplantation
	  ○ Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score.
	• Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
	  ○ Bilirubin, creatinine, INR and albumin values
	
	• Long term safety follow-up
	  ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1
	  ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of transplantation
	  ○ New ACLF episode will be summarized at Month 3 and Year 1

Study Assessment visits	<u>Study visits</u> During the screening and and treatment period, patients will be hospitalised. During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit). Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating

	HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given. on HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped. A study visit will be performed on Day 14 ± 2 days, including the evaluations listed below. On the other days during the hospital stay, patients will be followed-up according to usual practice. After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged. After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month). Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2). At the Month 12 study visit, patients will be invited to be included in the long term safety follow up study (4 years). <u>Study assessments</u> • All AEs up to Day 28 • All AESI up to Year 1 • Concomitant medication modifications up to Day 28 • Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6

	and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3, 6 and 12
	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ White Blood Cell count, Red Blood Cell count, platelets
	  ○ GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
	  ○ Creatinine, Urea or BUN
	  ○ CRP
	  ○ INR, aPTT
	  ○ Serum albumin, sodium, potassium,
	• Lipase: at screening
	• Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
	• Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
	• Protein C, Protein S, anti-thrombin III: at screening
	• Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab)
	• Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
	• Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab)
	• Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28
	• Chest x-ray,
	• Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
	• ECG: at screening (if not performed during same admission)
	• Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion
	Transjugular liver biopsy: – only data collection in case the biopsy has been done

	during the same admission or previously – no transjugular liver biopsy is required for the study protocol.A SMC will review safety data and advice on study conduct. Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion. In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw. In case of liver transplantation, a sample of the explanted liver will be collected if possible.
Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics. Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who receive at least one infusion. All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug

	Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

	The Report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Study Flow chart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	**Baseline**	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	**D21[b]**	**D28[c]**	**M2**	**M3**	**M6**	**M12**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

‡ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

1. BACKGROUND AND RATIONALE

1.1. ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Epidemiological studies indicate that there is an increasing prevalence of liver cirrhosis related to chronic infection by hepatitis C or B virus, alcohol consumption and non-alcoholic steatohepatitis worldwide (Murray *et al.* 2012). The natural course of cirrhosis is from compensated to decompensated disease. Decompensation is characterized by the development of major complications of liver disease (variceal bleeding, ascites, hepatic encephalopathy and bacterial infections) and is associated with poor prognosis. In addition to acute decompensation, ACLF is characterized by organ/system failure(s) (liver, kidney, brain, coagulation, circulation and/or lung) and high short-term mortality (33% at 28 days and 51% at 90 days). Approximately 31% of patients admitted to hospital for acute decompensation of cirrhosis present ACLF at admission (20%) or develop the syndrome during hospitalization (11%) (Moreau *et al.* 2013). Mortality rate depends on the number of failing organs as defined by the CLIF-SOFA score or the CLIF-OF score (a simplified version of the CLIF-SOFA score) (Table 1-1) (Moreau et al. 2013, Arroyo et al. 2015). Three grades define ACLF severity (Table 1-2). ACLF grade 1, defined as single kidney failure or single "non-kidney" organ failure with serum creatinine of 1.5-1.9 mg/dL and/or hepatic encephalopathy grade 1-2, is the most prevalent form of ACLF (15.8% of patients admitted at hospital with acute decompensation) and has a 28-day mortality rate of 23%. Patients with ACLF grade 2 (2 failing organs; prevalence 10.9%) have an intermediate prognosis (28-day mortality rate of 31%). Finally, ACLF grade 3 (with 3 or more organ failures) is the less frequent form of ACLF (4.4%) but shows extremely high mortality rates reaching 75% at 28 days.

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years, frequently alcoholics, in relation to a systemic inflammatory reaction due to bacterial infections, acute alcoholic liver injury or, in 40% of patients, to as yet unidentified precipitating events (Moreau et al. 2013). The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion, which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 or renal replacement
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by

translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may causes necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidences support the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety

profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properites of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013). Later, the technology of large-scale cell production was transferred to Promethera Biosciences which produce clinical batches of HHALPC in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs). In immunodeficient (SCID) mice transplanted with liver-derived progenitor cells, evidence of presence of human hepatocyte-like cells in the liver supported the biological plausibility of cell engraftment (Najimi *et al.* 2007; Khuu *et al.* 2011; Khuu *et al.* 2013). Short-term biodistribution assessed in rats using cells labeled with oxine ^{111}-Indium showed that cells concentrated in the liver (until 72 hours) (Tondreau et al. in preparation). Risk of tumor formation has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. *In vitro* studies showed that liver-derived progenitor cells present a low immunogenic phenotype (Sana *et al.* 2014). These cells have a pro-coagulant effect, similar to bone marrow derived MSCs, which may favor thrombosis. A study showed that concomitant treatment with an antithrombin activator or direct factor Xa inhibitor and direct thrombin inhibitor proved to be a particularly effective combination for controlling the procoagulant effects both *in vitro* and *in vivo* (Stephenne *et al.* 2012) (Please refer to the IB for more details).

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

Selected patients under the hospital exemption regulation were treated with liver-derived progenitor cells infused *via* the portal vein. In one of them, short-term biodistribution assessed using cells labeled with oxine ^{111}-Indium showed liver biodistribution of the cells (Sokal *et al.* 2013; Defresne *et al.* 2014).

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg)

(4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and

morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. This data lays the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

A hospital exemption treatment was conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated well this cell infusion, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, right ankle, and spine, and had disappeared from the lungs.

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell

activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For examples, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012). In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

The doses reported in publications on MSCs evaluation in immune-mediated diseases are varying usually between 0.5 to 3 millions cells/kg/infusion (Vandermeulen et al. 2014, Sharma et al. 2014). Single infusion or repeated infusions are reported. For example, remestemcel-L (Prochymal®, Osiris Therapeutics) was given IV at a dose of 2 x 10^6 MSCs/kg of body weight twice weekly for 4 consecutive weeks. Patients received all 8 infusions in the initial treatment plan by day 28. Infusions were administered at least 3 days apart (Kurtzberg et al. 2014). In some reports, higher doses were given, up

to 800 millions cells/infusion (~11 millions/kg/infusion) (Ra et al. 2011, Mayer et al. 2013, Lublin et al. 2014, Melmed et al. 2015). In inflammatory liver diseases, doses varying between 0.5 to 5 millions cells have been reported (Berardis et al. 2015). To be noted, the mode of administration was IV route and no concomitant administration of anti-coagulation medication was reported.

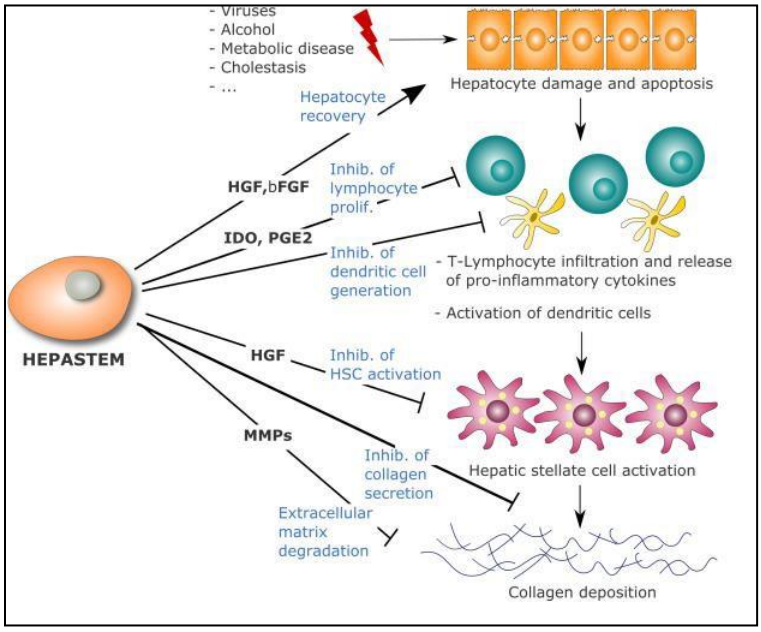
1.2.6. Pre-clinical immunomodulatory data of liver-derived progenitor cells

The first transcriptomics and secretomics tests performed on liver-derived progenitor cells grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models present important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.7. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing in the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.8. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts of 6 ACLF patients each. The study will include patients with ACLF grade 1 or 2 (excluding those with renal organ failure only, or those with circulatory or respiratory failure). It is planned to have a first group of 6 patients (cohort 1) being administered with 4 infusions of 250 millions cells each. Once this has been proven safe, a second group of 6 patients (cohort 2) will receive 4 infusions of 500 milions cells each. The infusions will be administered over 2 weeks with the first infusion started within a few days after patient's hospitalisation due to acute liver decompensation leading to ACLF. HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period.

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 6 patients, appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

HepaStem administration will be started rapidly after hospitalisation and will be completed within 2 weeks. This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of ACLF patients. As ACLF is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up for safety up 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining design of future efficacy clinical studies.

Study population

The selected adult population presents an expected mortality rate at short-term (28 days) close to 30%, which justify the use of a novel approach carrying potential short term benefits based on its immunomodulatory and anti-inflammatory properties. The short term mortality risk at 28 days is estimated close to 23% for ACLF grade 1 or 31% for ACLF grade 2. Patients with ACLF grade 1 with kidney

failure only will be excluded as their mortality risk at 28 days is actually close (< 20%) to that of patients with one (non-kidney) organ failure only (No ACLF) (Moreau et al. 2013). Patients with ACLF grade 3 will be excluded as they have a mortality rate reaching 75% at 28 days, making difficult to assess any safety or efficacy cell effect in this group. ACLF mainly occurs in cirrhotic patients aged around 50-60 years.

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 14 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 28 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 millions cells per infusion) is close to MSC doses given in other trials for immune mediated inflammatory diseases (Section 1.2.5), therefore, it is expected to show similar safety and efficacy profile. It corresponds also to the dose of liver-dirived progenitor cells administered via IV to the heamophila patient (see 1.2.4). The second selected dose represents a two-fold increase, still in the range of doses reported for MSCs. In addition, both doses are in the low range compared to HepaStem doses given in HEP001 paediatric study where administration of 500 millions cells per day was shown to be safe and well tolerated (see 1.2.3).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 UI/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damages nor any respiratory symptoms, before homing mainly in the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in ACLF patients, especially since it allows repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs have also a

procoagulant activity comparable to liver-derived progenitor cells (Stephenne et al. 2012), nevertheless literature report show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015). Furthermore, it has been established that hemostatic potential in patients with chronic liver disease is in a rebalanced status due to concomittant decrease in pro and anti-hemostatic drivers. In ACLF patients, the inflammatory process may trigger the unstable balance of hemostasis of cirrhotic patients to any of two states and may be manifested by either bleeding or thrombotic complications (Blasco-Algora et al. 2015). Thus an anti-coagulation may be contra-indicated in ACLF patients as they could be at risk of gastrointestinal hemorrhage, risks that may not be assessed by coagulation tests (prothombine time, INR, thrombin generation and thromboelastometry) (Lisman et al. 2012, Stravitz et al. 2012, Tripodi et al. 2009a, Tripodi et al. 2009b). Furthermore, bivalirudin use is not validated in cirrhotic patients. In order to mitigate risks of thrombosis in ACLF patients receiving HepaStem, several precautionary measures will be taken, as described in Section 5.5.1.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of two dose regimens of HepaStem in Patients with ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evalute clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

The study will recruit patients who are hospitalized for ACLF or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

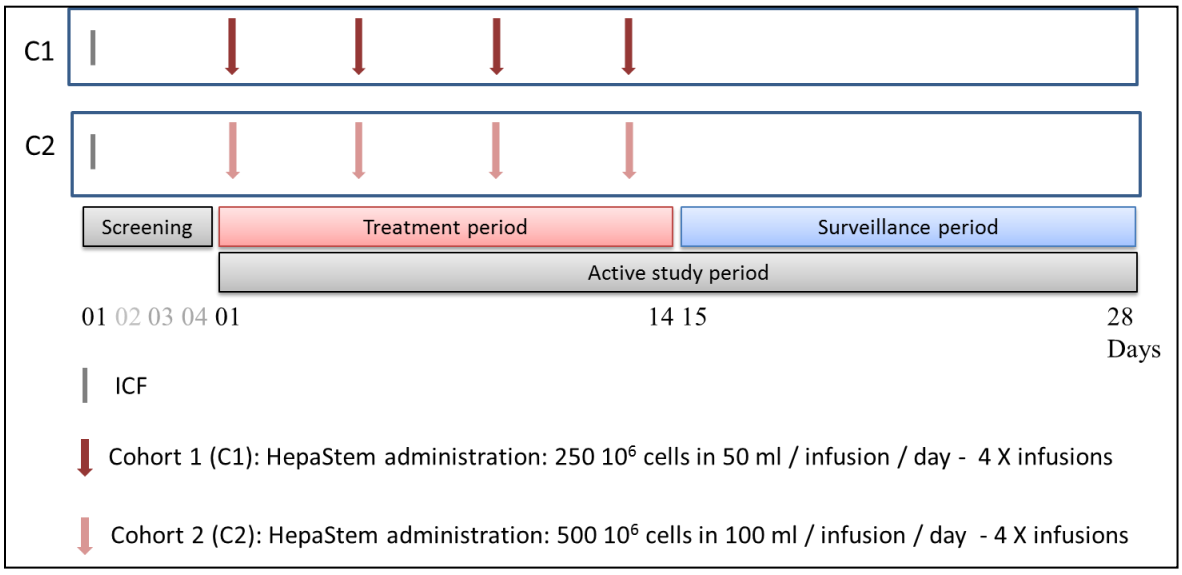
Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days), including a 2-week treatment period (± 2 days) and a 2-week evaluation period (± 2 days). The duration of the screening period plus the active period will last up to 32 days (± 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



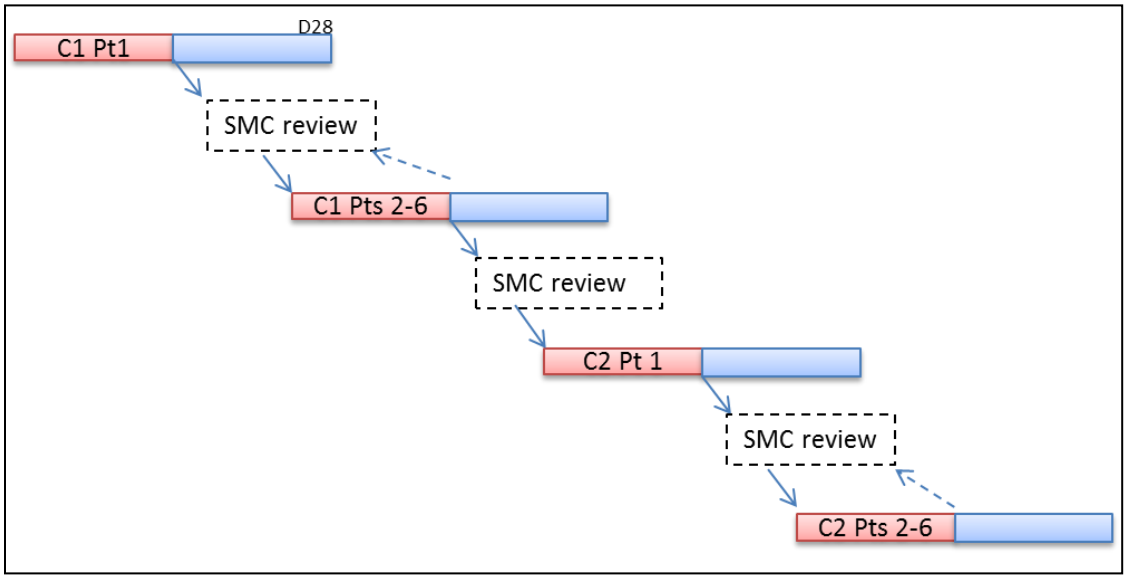
The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort. Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of Promethera members and external members (See Section 9.13):

- When the first cohort 1 evaluable patient will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

- When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advice on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

- When the first cohort 2 evaluable patient will have received HepaStem infusions (complete sheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advice to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advice on the enrolment approach for the next patients based on upcoming safety data.

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring Committe



The study assessments are described in Section 6.

Furthermore, the 3 first patients of each cohort will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete scheme or premature stop). The sequential approach will be under the control of Promethera (based on review of eligibility criteria by the medical monitor and HepaStem delivery). In case of safety signal, the SMC will be involved in the AEs review and evaluation, and the SMC will advise on further inclusion.

These measures (SMC meetings and sequential treatment for the 3 first patients in each cohort) will allow respecting the progress of dose levels with limited risk for the patients.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be invited to be followed-up in the long term safety follow up study (4 years).

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment, and in addition by SMC and the sponsor pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-ACLF score, MELD score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
  - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of transplantation
- New ACLF episode will be summarized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in 2 successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in 5 to 10 clinical centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. The hospitalisation unit will be adapted according to the medical status of the patient and the organization of study center hospital. Patients with a low CLIF-OF score will be more likely included in the hepatology department (standard or intermediate care unit), while the patient with high CLIF-OF score will more likely be included in the Intensive Care Unit.

Patients will remain hospitalised at least during the treatment period. During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Patients with ACLF at first evaluation post-admission or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
   N.B.: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

   - liver histology or
   - clinical and imaging examination (may include fibroscan).

4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions

ACLF grade 1 eligible subset:

- liver **failure** plus cerebral and/or kidney **dysfunction**

- renal **failure** plus cerebral **dysfunction**
- cerebral **failure** plus kidney **dysfunction**
- coagulation **failure** plus cerebral and/or kidney **dysfunction**

Or

ACLF grade 2 eligible subset:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfuncytions or failures are defined according to CLIF-C OF score as below

Diagnostic criteria of kidney and cerebral dysfunction

- kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL.
- cerebral: moderate impairment of brain function as defined by grade I-II HE based on West Haven criteria.

Diagnostic criteria of organ failures

- liver: serum bilirubin ≥ 12 mg/dL;
- kidney: serum creatinine ≥ 2 mg/dL or serum creatinine < 2 mg/dL because corrected by Terlipressine should be considered as an organ failure
- cerebral: grade III-IV HE based on West Haven criteria;
- coagulation: international normalized ratio [INR] ≥ 2.5

For both grades, patients with circulatory and/or respiratory failure are excluded (see exclusion criteria 6 and 7).

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.
   Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.
3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 48h.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.

5. Clinical evidence of aspergilus infection.
6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criteria.
7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
8. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period
9. MELD score > 35.
10. Previous organ transplantation and/or ongoing immunosuppressive treatments.
11. Postoperative-decompensation following hepatectomy.
12. Renal failure due to chronic kidney disease.
13. Clinically significant left-right cardiac shunt.
14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
18. Participation to any other interventional study within the last 4 weeks.
19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration for a patient are the following:

Transitory discontinuation:

- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem. Infusions may be restarted after recovery. If planned infusions are not performed within the 2 week treatment period (± 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, *i.e.*, thrombosis, anaphylactic shock, severe acute lung injury.

- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock, severe worsening of hepatic function.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAW CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No Patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. ACLF resolution or detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and

the syringe will be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least 2-day interval without infusion must be respected between infusion days.

In cohort 1, 250 millions cells in 50 ml will be administered on each infusion day, leading to a total of 1 billion cells. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes.

In cohort 2, 500 millions cells in 100 ml will be administered each infusion day, leading to a total of 2 billions cells. On each infusion day, 2 infusions of 50 ml reconstituted HepaStem suspension will be given. An infusion is expected to last 50 minutes. The two infusions can be given subsequently. HepaStem shall be reconstituted accordingly.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate or ICUs or standard units, depending on the medical status of the patient and the organisation of study center hospital. Regardless of the unit of hospitalization, patients will remain hospitalised at least during the treatment period, with a close monitoring of each patient. During HepaStem infusion, a continuous monitoring of the vital signs of the patient is required.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension have to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics and risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis, respiratory disorder as cells first transit to the lungs, hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the below mentioned risks, there might be other, at this time, unknown risks.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lungs disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect. **Number of cells administered per infusion will be maximum 500 millions cells and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min**. The lower dose regimen will be applied before the higher one. These doses are in the low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication (See Section 1).

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions. Any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion will be excluded from the study.

In case of clinical signs or suspicion of pulmonary embolism, the investigator should perform exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigraphy.

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Long Term Safety follow-up Period of 1 year. Thereafter, patients will be invited to be followed-up in a long term safety follow up study (4 years).

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and treatment period, patients will be hospitalised in intermediate or ICUs or standard units, depending of the severity of the patient disease.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of ACLF, significant background condition, relevant medications, and factor(s) triggering ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1. For both cohort 1 and cohort 2, patients will receive HepaStem on 4 infusion days spread over a 2-week period ($\pm$ 2 days); at least 2-day interval without infusion must be respected between infusion days. If planned infusions are not performed within this period, missed infusions will not be given.

On HepaStem infusion days (defined as Day 1, 4, 8, 12, to be adapted based on actual infusion day), before infusion, a physical exam, evaluation of vital signs, blood tests, a liver echography and Doppler, and evalution of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

During the infusion, the patient will be continuously monitored for early detection of any potential AEs.

A study visit will be performed on Day 14 $\pm$ 2 days, including the evaluations listed below.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

In case of any suspicion of AE, the investigator will perform the exams she/he evaluates as appropriate. In particular, any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement. In case of clinical signs or suspicion of pulmonary embolism, the investigator should perfom exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigrapy.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to Month 3 visit, all SAEs will be collected. After Month 3 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of transplantation, malignancies, new ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be included in the long term safety follow up study (4 years).

6.2.1. Study assessments

- All AEs up to Day 28
- All AESI up to Year 1
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-ACLF and MELD scores will be estimated from clinical and biological results: at screening, on Days 7, 14, 21, 28, Months 2, 3, 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets
    - GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening

- Protein C, Protein S, anti-thrombin III: at screening
- Thomboelastogram, thrombin generation test: at screening, Days 1, 4, 8, 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by luminex method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28
- Chest x-ray,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)
- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion
- Transjugular liver biopsy: – only data collection in case the biopsy has been done during the same admission or previously – no transjugular liver biopsy is required for the study protocol.

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

<u>Thromboelastogram and thromboglobulin generation tests</u>

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min. twice). The plasma will be colleted and put in cryotubes (min. 2 tubes containing each min. 500 microL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Hippocrate 10,
1200 Woluwe-Saint Lambert BELGIUM

Anti-HLA antibodies

Serum will be collected and frozen.

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Hippocrate 10 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHART

Table 6-1 Study Flowchart

	Screening Period	Active period							Follow Up period			
		Treatment Period					Surveillance Period					
Time	**Baseline**	D1[b]	D4[b]	D8[b]	D12[b]	D14[b]	**D21[b]**	**D28[c]**	**M2**	**M3**	**M6**	**M12**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	ǂ	ǂ	ǂ	ǂ	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-ACLF, MELD	X	«	«	«	«	X	X	X	X	X	X	X
Biological analysis												
WBC, RBC, Plt, CRP, Na^+, K^+, Alb, Creat, Urea/Bun	X	«	«	«	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«	«	«	«	X	X	X	X	X	X	X
Coagulation 2 : TEG, TG (central lab)	X	«	«	«	«	X	X	X				
Coagulation 3 : C-Protein, S-Protein, Anti-Thrombin III	X											
Virology status (HBS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Anti-HLA antibodies (Cl 1 and Cl2) (Central lab)	X							X		X	X	X
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma Cytokines (Central Lab)	X	«		«		X						
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X		X				
Chest X-Ray	©											
Cardiac US Doppler	©	≠										
ECG	©											
Blood culture or other fluid culture	¥											
Transjugular liver biopsy	O											
Investigational Product : HepaStem Infusions[a]												
Cohort 1 : Infusion of 250 Million cells/50 ml + Hydrocortison (100mg)		X	X	X	X							
Cohort 2: Infusion of 500 Million cells/100 ml +Hydrocortison (100mg)		X	X	X	X							
Concomitant medication & therapy		Continuously							Relevant			
Safety (**A**dverse **E**vents)		All AEs							AESI			

a) Infusion day: 4 infusions on 14 days period (± 2 days) with at least 2 day interval without infusion.

Hydrocortisone given 15-30 min before HepaStem infusion

b) Day to be adpated, cfr remarque a)

c) ± 2 days; End of Active Period visit

« Before each infusion .

© if not aleady performed during same admission; if already performed, results collected

‡ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

O Optional; if already performed during same admission, results collected

≠ : cardiac US to be performed after infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome
- Transplantation and outcome of the transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

Possibly

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probable

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definite

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

Moderate, when interfering with ADL;

Severe, when impeding with ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SAR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of 2 dose regimens of HepaStem given in 2 subsequent cohorts each of 6 hospitalized ACLF patients.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2.

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics.

Continuous variables will be summarized by reporting the number of Patients, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

The transplant-free and overall survival will be estimated for each treatment dose and overall using Kaplan-Meier curves.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences must "verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection".

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator's Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient's further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission

for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of Promethera members including medical monitor, pharmacovigilance representative, clinical representive and external members with expertise in liver disease or other relevant medical fields. The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.
2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.
    - When the first cohort 1 evaluable patient will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 1 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data.
    - When 6 cohort 1 evaluable patients will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will advise on the enrolment of the first cohort 2 patient. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.
    - When the first cohort 2 evaluable patient will have received HepaStem infusions (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC may advise to enrol the next 5 patients of cohort 2 or to continue a stepwise enrolment approach. In this case, the SMC will advise on the enrolment approach for the next patients based on upcoming safety data.
5. The SMC will review severe thrombotic events assessed as related to HepaStem administration by the investigator.

6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- One or more biostatisticians
- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists may be invited to participate as non-voting members at any time if additional expertise is desired.

The frequency of SMC meetings will depend on several factors including the rate of enrolment, completion of five patients of the low dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.

Organ / System	Sub-score = 1	Sub-score = 2	Sub-Score = 3
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL or renal replacement
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2	>300	≤300 - > 200	≤200
or SpO2/FiO2	>357	>214- ≤357	≤214

Arroyo et al. 2015

11.2. MELD SCORE

MELD Score based on
- serum Creatinin
- serum Bilirubin and
- INR

Chung et al. 2012

11.3. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 20 December 2016

Version Number: 2.1

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____

_____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 20 December 2016

Version Number: 2.1

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competente Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

_____ _____

Joëlle Thonnard, Director Clinical and Medical Affairs Date (dd/mm/yyyy)

_____ _____

Etienne Sokal, MD Chief Innovation & Scientific Officer Date (dd/mm/yyyy)

_____ _____

Silver Ocean Ventures SAS, CEO, represented by John Tchelingerian Date (dd/mm/yyyy)

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 3.1 – 21 Mar 2017

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Innovation & Scientific Officer John Tchelingerian, PhD – Chief Executive Officer Nancy Veulemans, Ir – Vice President Medical and Clinical Affairs Joëlle Thonnard, MD- Head of Medical Affairs and New Indication
MEDICAL MONITOR	Joëlle Thonnard, T.+32.471.563.423
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LIST OF ABBREVIATIONS

AD	Acute Decompensation
ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SADR	Serious Adverse Drug Reaction
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 3.1 - 21 Mar 2017
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Twelve (12) evaluable Patients
Number of Centers	5-10 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total 5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 ce
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 ce
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 ce

The next 3 patients enrolled will complete the first cohort and will receive a lower dose following SAEs observed in patient 2 and patient 3.

Six other patients will be enrolled in cohort 2.

The 3 first patients of cohort 2 (cohort 2a) will receive twice the dose compared to cohort 1b.

The 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

The statistical analysis will take into consideration the different doses applied.

Study periods

The study will recruit cirrhotic patients who are hospitalized for ACLF or Acute Decompensation at risk of developing ACLF

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Patients with Acute Decompensation of cirrhosis at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

The study is divided in the following periods: screening period, active study period divided in treatment period and evaluation period, and long-term safety follow-up.

Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days).The duration

of the screening period plus the active period will last up to 32 days (± 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Various dose regimens of HepaStem will be given, which differ in the amount of cells per infusion and/or in the number of infusions.

The 3 first patients received the dose regimen of 250.10^6 cells per infusion – this represents approximately $3.5.10^6$ cells/kg bodyweight in the first cohort. (cohort 1a)

The next three patients in cohort 1 (cohort 1b) will receive a lower dose (minimum ten times lower) in a single infusion ($0.25.10^6$ cells /kg bodyweight with a maximum of 25.10^6 cells per infusion).

The 3 next patients in cohort 2 (cohort 2a) will receive twice the dose of the patients in cohort 1b ($0.5.10^6$ cells/kg bodyweight with a maximum of 50.10^6 cells per infusion).

The 3 next patients in cohort 2 (cohort 2b) will receive up to 2 doses of $0.5.10^6$ cells/ kg bodyweight 1 week apart ($0.5.10^6$ cells/kg bodyweight per infusion with a maximum of 50.10^6 cells per infusion).

Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of external members and Promethera members.

As a minimum, the safety data will be reviewed by the SMC at the following timepoints :

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received the HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patients in the next cohort.

In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

	More specifically, based on the patients' parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg bodyweight) instead of a single administration of $0.5.10^6$ cells/kg bodyweight). Furthermore, the 3 patients of each cohort will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete sheme or premature stop). Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period. After completion of this study, patients will be invited to be followed-up in the Patient Registry (5 years).
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and thereafter for an additional period of 1 year in the long term safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at $50x10^6$ cells/ml equivalent to $250x10^6$ cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent. In cohort 1a, 50 ml was given per infusion. For cohorts 1b, 2a and 2b, the volume of HepaStem administered will be adapted to the patient's bodyweight.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on the patient's status. The infusion rate shall not exceed 1 ml per min. For cohort 1, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion had to be respected between infusion days. The *Planned* schedule was : in cohort 1a, 250 million cells in 50 ml were administered on each infusion day, leading to a total of 1 billion cells ,if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone was expected to be given 15 to 30 min before

	each HepaStem infusion. The *Actual* schedule is: in cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2a: 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).
s	Inclusion criteria: 1. Adult aged between 18 and 70 year old. 2. Informed Consent. N.B: In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement. 3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan). 4. Patient with Acute Decompensation of cirrhosis

	5. Serum total Bilirubin ≥ 6 mg/dL (≥100 umol/L) 6 The INR measurement has to be : 1.2 ≤ INR < 2
Eligibility - Exclusion Criteria	<u>Exclusion criteria</u>: 1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam. 2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion. 3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion. 4. Variceal banding or sclerosis within 4 weeks before the infusion. 5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 6. Clinical evidence of Aspergillus infection. 7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion. 8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation. 9. Coagulation disorders defined as : • INR ≥ 2 • Fibrinogen < 100 mg/dL • Platelets < 50.000/mm^3 10. Major invasive procedure within 1 week before the infusion (including but not limited to transjugular liver biopsy) 11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period. 12. MELD score > 30. 13. Previous organ transplantation and/or ongoing immunosuppressive treatments. 14. Postoperative-decompensation following hepatectomy. 15. Renal failure due to chronic kidney disease. 16. Clinically significant left-right cardiac shunt. 17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of

	severe anaphylactic reactions. 18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. 19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 21. Participation to any other interventional study within the last 4 weeks. 22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisousness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28. The relationship will be assessed based on investigator assessment, and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline. <u>Secondary Endpoints</u>: • Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Mortality ○ Liver transplantation ○ Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score, Child Pugh score. • Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Bilirubin, creatinine, INR and albumin values • Long term safety follow-up ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1 ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of the transplantation, New ACLF episode will be summarized at Month 3 and Year 1
Study Assessment visits	<u>Study visits</u>

	During the screening and treatment period, patients will be hospitalised. During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit). Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. On HepaStem infusion days,, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, TEG (if already performed as part of the clinical routine and up to investigator's judgment), coagulation factors (intrinsic and extrincsic pathway), a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day (even if HepaStem infusions are prematurily stopped). The careful evaluation of these parameters will allow or will not allow the infusion (see section 4.4 – Criteria for treatment discontinuation). On the other days during the hospital stay, patients will be followed-up according to usual practice. A study visit will be performed on Day 4, 8, 12 and 14 ± 2 days post 1st infusion, including the evaluations listed below. After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged. After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks),

and Year 1 (± 1 month).

Up to Day 28 visit, all SAEs will be collected. After Day 28 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of the transplantation, malignancies, new AD and/or ACLF episode, AEs assessed by the investigator as possibly related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be included in the Patient Registry (5 years).

Study assessments

- All AEs up to Day 28
- All AESI up to 1 Year.
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD score (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12,, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
    - GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium
- Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.

- INR
- aPTT
- fibrinogen
- D-Dimers
- TEG (optional, only if measurement can be done locally and up to investigator's judgment)

- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion.
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and Aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening. In case of deterioration of the coagulation, these measurements will be repeated.
- Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2b), 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also at M12.Chest x-ray : at screening (if not performed during same admission) and at M12,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission). Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdrawal from study, an end of study visit should be perfomed if possible at the time of study withdrawal.

In case of liver transplantation during the course of the study, a sample of the explanted liver will be collected if possible.

Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem is administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist. The 3 first patients infused (cohort 1a) received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. 2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage to a new low dose. (see section 1)). The next 3 patients will receive a lower dose of HepaStem and the next 6 patients (new high dose cohort) will receive the higher dose. The total sample size consideration remains unchanged with a total of 12 patients.
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who received at least one infusion. All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications

	and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of the transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The Report will be updated after these patients will have completed the 3 month

	follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Period	**Screening Period**	**Active period**							*Long term follow-up*			
	Baseline	**Treatment Period**					**Surveillance Period**					
Time	**Over 1-4 days prior D1**	Infusion D1	D4[b]	D8[c]	D12[b]	D14[b]	**D21[b]**	**D28[b]**	**M2[d]**	**M3[d]**	**M6[d]**	**M12[e]**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	↔	↔	↔	↔	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	«%	«	«%	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«+	«	«+	«	X	X	X	X	X	X	X
Coagulation 2 : C-Protein, S-Protein, Anti-Thrombin III	X§											
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen, D-Dimers, TEG®		+		+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	«		«		X		X				
TEG, TG	X	*	X	*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X	X	X				X
Chest X-Ray	©											X
Cardiac US Doppler	©	X										X
ECG	©											
Blood culture or other fluid culture	A											
~~Transjugular liver biopsy~~	~~O~~											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶ cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg)		X[a]										
Cohort 2a : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg)		X[a]										
Cohort 2b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg)		X[a]		X[a]								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a) Hydrocortisone given 15-30 min before HepaStem infusion

b) ± 2 days
c) ± 2 days with at least 7 days interval without infusion
d) ± 2 weeks
e) ± 1 month
« Before each infusion .
A : If already performed during same admission, results collected
% : On infusion day, platelets measurement to be performed prior and post infusion at 4h, 24h, 48h and 72h.
© if not already performed during same admission; if already performed, results collected
ǂ before, during, after each infusion
¥ If applicable (If already performed during same admission, results collected)
≠ : cardiac US to be performed after infusion
@ : Optionnal, only if measurement can be done locally and up to investigator's judgment
+ : On infusion day : prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion (frequency of these exams can be increased up to the investigator's judgment.)
* : Before infusion and 4h post infusion
AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation
& : Prior and 24h after infusion
§ : In case of deterioration of the coagulation, these measurements will be repeated.

1. BACKGROUND AND RATIONALE

1.1. CIRRHOSIS, ACUTE DECOMPENSATION AND ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Cirrhosis is a progressive chronic liver disease characterized by diffuse fibrosis, severe disruption of the intrahepatic venous flow, portal hypertension and liver failure. The course of cirrhosis is divided into two stages. Compensated cirrhosis defines the period between the onset of cirrhosis and the first major complication. During this period, which is relatively long in most patients (>10 years), symptoms are absent or minor, but liver lesions and portal pressure steadily progress. The term decompensated cirrhosis defines the period following the development of ascites (that is, the accumulation of large amounts of fluid within the peritoneal cavity), variceal haemorrhage and/or hepatic encephalopathy. This period is associated with short-term survival (3–5 years). It is increasingly evident that patients rarely die as a consequence of an end-stage irreversible destruction of the liver. Rather, in most patients, the cause of death is an acute deterioration in their chronic clinical condition promoted by a precipitating event — a syndrome termed acute-on-chronic liver failure (ACLF) (Arroyo et al. 2015).

It is of note that definitions on ACLF may differ worldwide. Given the heterogeneity and the importance of identifying patients four major societies/organisations have provided working definitions (APASL, NACSELD, WGO and EASL-CLIF). The common definition of ACLF is 'a syndrome characterised by acute decompensation of chronic liver disease associated with organ failure(s) and high short-term mortality'. According to the CLIF-ACLF definition developed based on the CANONIC study, ACLF is a recognised syndrome characterised by acute decompensation of cirrhosis associated with the failure of one or more organs and, in the more severe cases, system failure. The organs and systems most likely to fail are the liver, kidney, brain, coagulation, circulation and/or lungs. Patients have a high short term mortality of over 15 % at 28 days (Hernaez R et al, 2017). In the CANONIC study approximately 31% of patients admitted to a hospital for Acute Decompensation (AD) of cirrhosis had ACLF at admission (20%) or developed the syndrome during hospitalisation (11%). The common causes of acute decompensation of liver function included bacterial infections, alcoholic hepatitis, and gastrointestinal hemorrhages, but, in more than 40 % of patients, no precipitating event was identified (Moreau et al. 2013). Among patients with Acute Decompensation (AD), subgroups were identified as being at higher risk of progressing to full blown ACLF and thus at higher mortality risk (Arroyo et al. 2015).
Different grading/scoring systems have been developed in order to better determine prognosis and effectiveness of intervention and care. (Hernaez R et al, 2017).

In daily practice, MELD and Child Pugh scores are still strongly relied on to guide clinical care.

The Model for End-Stage Liver Disease, or MELD, is a scoring system for assessing the severity of chronic liver disease. This score is used by the United Network for Organ Sharing (UNOS) and Eurotransplant for prioritizing allocation of liver transplants. New MELD uses the patient's values for serum bilirubin, serum creatinine, sodium and the international normalized ratio for prothrombin time (INR) to predict survival.

Mortality and MELD score are linearly correlated amongst patients with end-stage liver disease listed for OLT with 3-month mortality estimated to be 4%, 27%, 76%, 83%, and 100% for MELD scores of <10, 10–19, 20–29, 30–39, and 40 or more respectively.

The Child–Pugh score is used in clinical practice to assess the prognosis of chronic liver disease, mainly cirrhosis. It was previously used for prioritizing allocation of liver transplants. The score employs five clinical measures of liver disease: total bilirubin, serum albumin, prothrombin time, ascites and hepatic encephalopathy. Each measure is scored 1–3, with 3 indicating most severe derangement. This leads to three Classes with one year overal survival of 100% for Class A, 81% for class B and 35% for class C. (see 11.6)

ACLF has been defined by the CLIF research consortium into four grades based on retrospectively fitting data on severity linked to mortality score (Moreau et al. 2013) (Table 1-1 and Table 1-2)

- ACLF grade 0 concerns 69.1 % of patients admitted to hospital with acute decompensation. The group is defined as no organ failure, single "non kidney" organ failure (ie, single failure of the liver, coagulation, circulation, or respiration) who had a serum creatinine level < 1.5 mg/dL and no hepatic encephalopathy, or as single cerebral failure with a serum creatinine level < 1.5 mg/dL. These patients have a 28-day and 90-day mortality rate of 4.7% and 14% respectively.
- ACLF grade 1 concerns 15.8 % of patients admitted to hospital with acute decompensation. The group is defined as single kidney failure or single non-kidney organ failure with an organ dysfunction (kidney or brain) and has a 28-day mortality rate of 23 %.
- Patients with ACLF grade 2, defined as two failing organs (10.9 % of patients admitted to hospital with acute decompensation) has an intermediate prognosis (28-day mortality rate of 31%).
- Finally, ACLF grade 3, defined as three or more organ failures (4.4 % of patients admitted to hospital with acute decompensation) has extremely high mortality rates, reaching 75 % after 28 days.

Among patients hospitalised with acute decompensation (AD) (pre ACLF according to the CLIF criteria but ACLF according to other classification systems), an analysis revealed five independent variables including age, serum sodium, white cell count, creatinine and INR as useful for defining a scoring system. The high-risk group (CLIF-C AD score > 60) and intermediate risk group (CLIF-C AD score 46-59) respectively have a 3-month mortality of over 30 % and between 2-30 %. The low risk AD group has a 3-month mortality below 2 % (Arroyo et al. 2015).

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years.The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

Cirrhotic patients with acute decompensation can only receive supportive treatments, such as antibiotics in case of infection, lactulose in case of encephalopathy, terlipressin and albumin in case of hepatorenal syndrome. However, at this moment, there are no treatments available to stop the inflammatory cascade often accompanying the acute decompensation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion,

which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 , renal replacement or use of vasopressors for Hepatorenal syndrome indication
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may cause necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver transplant rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

Conclusion on patient population: Based on this information, Promethera Biosciences proposes that the patient population is defined as cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 µmol/L) and MELD < or = 30.

Patients should have coagulation parameters within the ranges below:

- INR ≥ 1.2 and < 2
- Fibrinogen ≥ 100 mg/ dL
- Platelets ≥ 50.000/mm3

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidence supports the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properties of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells (similar cells to HHALPC) were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013).

Later, the technology of large-scale cell production was transferred to Promethera Biosciences where clinical batches of HHALPC are produced in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs).

Toxicology *in-vitro or in-vivo* studies aiming to demonstrate the safety, tolerability and tumorigenicity aspect of HepaStem were conducted. *In vivo* studies were performed in rats and mice. They included one study to assess the safety of the intravenous mode of administration. Two studies specifically assessed the risk of tumor formation as this risk has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. An *in vitro* study the pro-coagulant activity of HepaStem was confirmed (Please refer to the IB for more details).

In addition, *invitro* studies show that HepaStem cells express variable immunomodulatory surface markers of interest and have immunomodulatory functional effects: HepaStem inhibits the proliferation of activated T-lymphocytes and blocks the maturation of monocytes (see Section 1.2.6). Furthermore, 6 *in-vivo* studies were conducted with HepaStem evaluating the immunomodulatory properties using the IV route of administration and mainly doses of 12.5 10^6 cells/kg. No safety signal was detected based on these *in vivo* studies.

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg) (4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few

days. None did undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. These data laid the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

Based on literature data and Promethera experiences, it can be concluded that liver-derived MSCs, including HepaStem have a pro-coagulant activity. This pro-coagulant activity is also expressed by other MSCs. The pro-coagulant activity might be linked to tissue factor expression, an activator of the coagulation cascade. The procoagulant effect could be modulated by the concomitant administration of bivaluridin during HepaStem infusion in UCD clinical trials in order to prevent, mainly, anticipated thrombotic events. Very high cell doses have been administered intra-portally in the UCD studies in which thrombotic events only occurred at high doses (range: 115 million to 4,1 billion total cells were administered in the portal vein as a split dose in 1 to 10 infusions spread over 1 to 4 consecutive days). Bivaluridin will not be used in the ACLF clinical study as its use has not been validated for late stage cirrhotic patients. Contrary to patients with urea-cycle disorders, coagulation disturbances are common in the late stage chronic cirrhosis population and are linked to liver insufficiency. (see 1.2.5)

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

In a first-in man cohort,conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells (ADHLSC, similar cells to HHALPC) infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated this cell infusion well, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6

cells repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, spleen, right ankle, and spine, and had disappeared from the lungs. This is in line with the bio-distribution of another type of MSCs administrated in patients (BM-derived MSCs) that demonstrate a similar bio-distribution, with a first pass through the lung; within 24 hours, cells are mainly found in liver, spleen, kidneys and other inflamed areas, by 48 hours, more pronounced presence in the liver is observed. (NDS dossier remestemcel-L, Health Canada).

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For example, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012).

In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

It is clear from consultation of literature publications on cell therapy administration in advanced liver disease including decompensated liver cirrhosis and ACLF that doses of cell therapy protocols tended to be lower as compared to the normal range administered to patients in other immune-modulatory or anti-inflammatory protocols.

The doses and regimens administered to treat patients with chronic liver diseases, range from 0.03 over 0.5 to 1 million MSC cells/kg bodyweight. Different regimens were applied with repeated dosing up to 3 times for the lowest doses (0.03; 0,05 and 0,5 million cells/kg BW repeated 3 times). Most protocols administered the cell infusion intravenously although also other routes of administration were investigated such as intra-splenic, hepatic artery, intrahepatic, intra-lesional route of administration or central venous catheter into the femoral vein. (Berardis et al. 2015)

Based on the literature review, MSC administration is considered to be safe due to the lack of reports of significant adverse effects in the above studies, although a marked heterogeneity was observed among studies with regard to injection dose, frequency of injection, cell source, delivery route and study design. Most of these early studies reported improvements in liver function, ascites and encephalopathy.

In the first cohort of 3 patients in the HEP001 study the lowest dose (12.5×10^6 cells/kg) of the range of doses administered safely in previous studies of HepaStem in urea cycle disorder (UCD) and Crigler Najar pediatric patients was used to determine the dose in the HEP101 protocol. The (low) dose proposed (250

million cells/ infusion; ie. 3.5 10^6 cells/kg BW/infusion) was a reduction of 4x of the lowest dose tested previously (in the HEP001 protocol) and it was thought that it could be safely administered in cirrhotic patients. Additionally, the number of cells administered per infusion would be limited, similar to MSC doses given in immune mediated inflammatory diseases. In retrospect, it was clear that adaptation to the dose level similar to other MSCs given in immune-mediated inflammatory diseases was inadequate and did not take the specific case of severely ill chronic cirrhotic patients with acute decompensation into account.

Therefore, it seems that using a careful approach starting with doses commonly used in reported studies of decompensated cirrhosis and ACLF patients and published as being safe, appears to be an acceptable approach. Also, dose escalation to a maximum of 1.0 million cells/kg BW should be feasible based on a repetitive dosing schedule. In case of repetitive dosing, the doses will be given weekly, which allows time in case of fibrinolysis for the parameters to be corrected and return to normal. (please refer to rationale for changes)

1.2.6. Pre-clinical immunomodulatory data of liver-derived progenitor cells

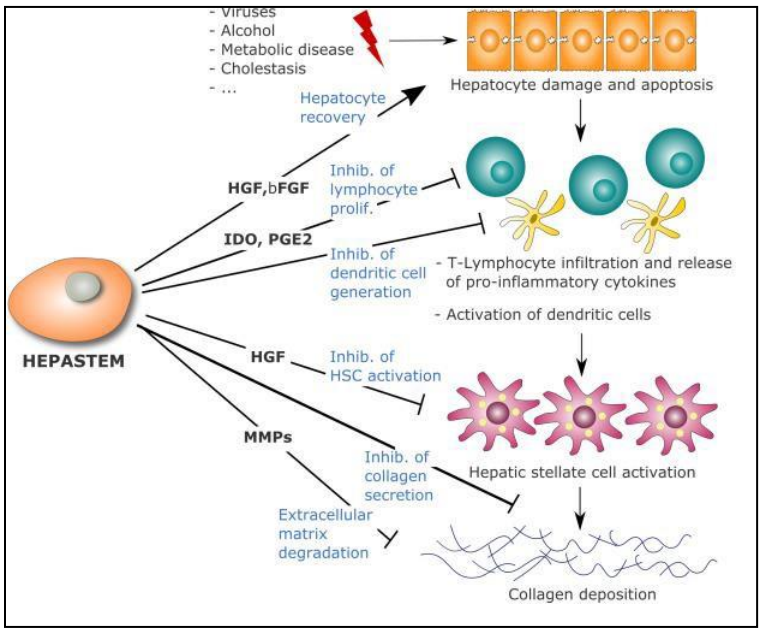
The first transcriptomics and secretomics tests performed on liver-derived progenitor cells (similar cells to HHALPC) grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (similar cells to HHALPC) (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of

HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models presents important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.7. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing to the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed

in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.8. Expected Benefits of HepaStem

Proposed mechanisms of action: after intravenous administration of HepaStem, cells are expected to circulate into the blood network where they can exert a systemic immunomodulatory action. At the same time, they have a main homing into the liver where they can thus also exert some important local immunomodulatory effects. They are expected to play their immunomodulatory roles through direct cell-to-cell interaction and through paracrine effects via the various cytokines, chemokines, MMPs and growth factors they may secrete. HepaStem could affect monocytes and DC recruitment, differentiation, maturation and function through cell contacts or paracrine signalling. HepaStem could also alter the proliferation and activation of T-lymphocytes that are another dysregulated cell type of the immune system in ACLF. In addition to modulate the behaviour of immune cells, HepaStem could modulate the proliferation and activation of hepatocytes and hepatic stellate cells and thus their secretory profiles, helping in this way the liver function recovery. The current *in vitro* and *in vivo* data, based on the scientific literature, and sponsor *in vitro* results, support all these potential immunomodulatory effects of HepaStem in ACLF patients.

Proposed clinical significant benefit: by these combined effects, HepaStem could play a favourable role in restoring an immunological balance in ACLF patients or patients at risk of ACLF, improving organ failure scores, improving clinical status, possibly leading to a resolution of this acute event and demonstrating improvement of transplantation free survival.

Considering the unmet medical need: i. the emergency to treat cirrhotic patients with Acute Decompensation (pre-ACLF or ACLF) due to the high mortality rate; ii. the shortage of healthy donors and the need of livers in the context of liver transplantation; iii. Concerns raised recently regarding artificial liver support; and iv. the mechanism of action of HepaStem, we can say that all these factors are in favour of a promising favourable benefit/risk balance for HepaStem. The exact profile of which patients will benefit most is under investigation, and also subject of this safety study.

1.2.9. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total. The study will include patients with an acute decompensation of cirrhosis and with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 30,excluding patients with circulatory,respiratory failure or severe coagulations disorders). It is planned to have a first group of 6 patients (cohort 1) being administered with the low dose.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between

infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 patients experienced an episode of severe bleeding. Therefore, it has been decided to reduce the dose in the low dose cohort to $0.25.10^6$ cells/kg bodyweight with a maximum of 25.10^6 cells in a single infusion. A reduction of minimum 10 times the dose previously used.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

Once this has been proven safe, a second group of 3 patients (cohort 2a) will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 6 patients, appears to be an acceptable approach in patients with ACLF or with acute decompensation at risk of developing ACLF for whom no specific therapeutic or curative treatment exist. (See section 1.1)

HepaStem administration will be started rapidly after hospitalisation and will be completed within 1 day (cohort 1b) or within 1 week (cohort 2b). This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of patients with or at risk of developing ACLF. As ACLF and/or Acute Decompensation of cirrhosis is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction

or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up for safety up 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining the design of future efficacy clinical studies.

Study population

The patient population is defined by cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 30 (see section 1.1).

Patients should have coagulation parameters within the ranges below:

- INR ≥ 1.2 and < 2
- Fibrinogen ≥ 100 mg/ dL
- Platelets ≥ 50.000/mm3

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 14 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 28 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 million cells per infusion) is close to MSC doses given in other trials for immune-mediated inflammatory diseases (Section 1.2.5), therefore, it was expected to show a similar safety and efficacy profile. It corresponded also to the high dose of liver-dirived progenitor cells (similar cells to HHALPC) administered via IV to the heamophila patient (see 1.2.4).

Due to the severe bleeding that occurred in 2 of the 3 patients that received 250 10^6 cells (50 mL of HepaStem) per infusion, the next selected dose (low dose cohort 1b) will be reduced to 0.25.10^6cells/kg bodyweight (with a maximum of 25.10^6 cells per infusion) administered in a single infusion (at least a 10x reduction of the dose administered in cohort 1a).

The second selected dose represents a two-fold increase from the dose in cohort 1b (0.5.10^6cells/kg bodyweight (with a maximum of 50.10^6 cells per infusion) still in the range of doses reported for MSCs and more in the range of doses administered in the specific case of severely ill chronic liver disease

patients with ACLF and acute decompensation of cirrhosis. (see1.2.5)). (for additional information, please refer to the rationale for changes).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 IU/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damage nor any respiratory symptoms, before homing mainly to the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF , it also can allow repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs also have a procoagulant activity comparable to liver-derived progenitor cells (similar cells to HHALPC) (Stephenne et al. 2012), nevertheless literature reports show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015).

However, liver failure results in a state of “rebalanced hemostasis” marked by a decrease in both pro-coagulation and anticoagulation factors. Patients with severe liver disease are not auto-anticoagulated. In essence, patients with severe liver disease, acute and/or chronic, have a tenuous rebalanced hemostasis that is easily perturbed by various disease states and concomitant medications and invasive procedures. Bleeding events including severe forms are common in these end-stage liver disease patients. The events of epistaxis and bleeding from puncture sites that occurred in 2 patients in cohort 1a (in retrospect a high dose in late stage cirrhotic patients), have been recognised in the literature as case reports. It was also stated that epistaxis as an overlooked cause of massive haematemesis in cirrhosis should be added to the list of upper GI bleeding.(Johal et al 2003). Hence, cirrhotic patients including ACLF patients are at increased risk of bleeding or thrombosis. Therefore dose reduction from normal ranges applied in other immune-modulatory diseases, modification of inclusion criteria and increased surveillance of liver and coagualation parameters is indicated.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of different regimens of HepaStem in cirrhotic patients presenting with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total.

5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next 3 patients enrolled will complete the first cohort and will receive a lower dose following SAEs observed in patient 2 and patient 3.

Six other patients will be enrolled in cohort 2.

The 3 first patients of the cohort 2 (cohort 2a) will receive twice the dose compared to the cohort 1b.

The 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

The study will recruit patients who are hospitalized for Acute Decompensation of cirrhosis and/or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

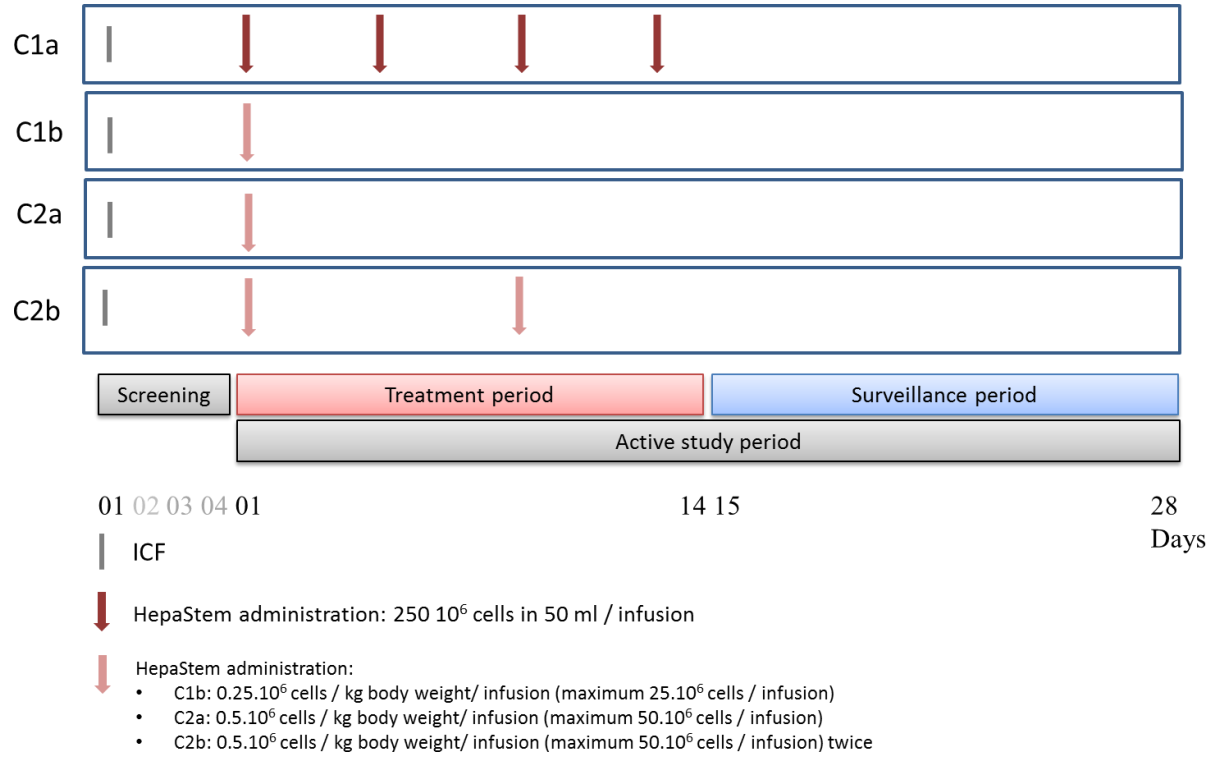
Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days ($\pm$ 2 days). The duration of the screening period plus the active period will last up to 32 days ($\pm$ 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



Planned schedule:

For cohort 1a, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion must be respected between infusion days.

In cohort 1a, 250 million cells in 50 ml were administrated on each infusion day, leading to a total of 1 billion cells if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion.

Actual schedule:

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

Planned schedule:

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be

adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

For cohort 2a: 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation

Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of external members and Promethera members (See Section 9.13):

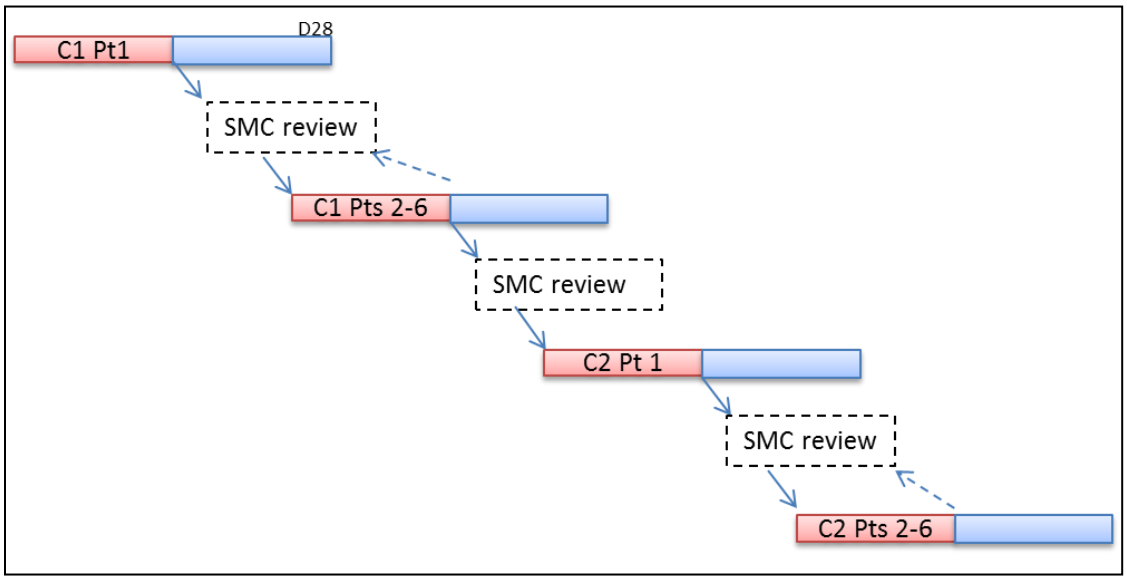
In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patients' parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a single administration of $0.5.10^6$ cells/kg body weight).

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring Committe



The study assessments are described in Section 6.

Furthermore, the 3 first patients of each cohort will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete scheme or premature stop). The sequential approach will be under the control of Promethera (based on review of eligibility criteria by the medical monitor and HepaStem delivery). In case of safety signal, the SMC will be involved in the AEs review and evaluation, and the SMC will advise on further inclusion.

These measures (SMC meetings and sequential treatment for the 3 first patients in each cohort) will allow respecting the progress of dose levels with limited risk for the patients.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be invited to be followed-up in the Patient Registry (5 years).

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

The relationship will be assessed based on investigator assessment and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
  - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of the transplantation
- New ACLF episode will be sumamrized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in 5 to 10 clinical centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days ($\pm$ 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. The hospitalisation unit will be adapted according to the medical status of the patient and the organization of study center hospital. Patients with a low CLIF-OF score will be more likely included in the hepatology department (standard or intermediate care unit), while the patient with high CLIF-OF score will more likely be included in the Intensive Care Unit.

Patients will remain hospitalised at least during the treatment period. During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Cirrhotic patients with Acute Decompensation at risk of developing ACLF at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
   N.B: In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

   - liver histology or
   - clinical and imaging examination (may include fibroscan).

4. Patient with Acute Decompensation of cirrhosis

5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)

6. The INR measurement has to be : $1.2 \leq INR < 2$

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.

   Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.
3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion.
4. Variceal banding or sclerosis within 4 weeks before the infusion
5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
6. Clinical evidence of Aspergilus infection.
7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion.
8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
9. Coagulation disorders defined as :
   - INR ≥ 2
   - Fibrinogen < 100 mg/dL
   - Platelets < 50.000/mm^3
10. Major invasive procedure within the week before the infusion (including but not limited to tranjugular liver biopsy)
11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.
12. MELD score > 30.
13. Previous organ transplantation and/or ongoing immunosuppressive treatments.
14. Postoperative-decompensation following hepatectomy.
15. Renal failure due to chronic kidney disease.
16. Clinically significant left-right cardiac shunt.
17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.

20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
21. Participation to any other interventional study within the last 4 weeks.
22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration for a patient are the following:

- **Transitory discontinuation:** Coagulation disorders considered as significant (INR ≥ 2, Fibrinogen < 100 mg/dL, or Platelets < 50.000/mm^3) by the PI prior to each infusion should preclude the administration of Hepastem.
- Absence of portal vein flow prior to the infusion should preclude the administration of Hepastem.
- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.

Infusions may be restarted after recovery. If planned infusions are not performed within treatment period (± 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock, severe worsening of hepatic function.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAWAL CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator

- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be

reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).
- The exact dosage (volume) of Hepastem infused to the patient will be calculated based on the weight of the patient on the day of infusion (0.25.10^6 cells per kg bodyweight with a maximum of 25.10^6 cells/infusion (5 mL) for cohort 1b or 0.5.10^6 cells per kg bodyweight with a maximum of 50.10^6 cells/infusion (10 mL) for cohort 2.)
- As the exact volume to infused can be low (depending on the patient's weight), it is recommended to flush after the infusion physiological solution (NaCl 0,9%) to ensure that all the product is infused.

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the

mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and the syringe will be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells per infusion). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapated accordingly. A single infusion is expected to last minimum 5 min (for 5 mL of reconstituted Hepastem).

For cohort 2a and 2b: 3 patients will receive HepaStem in a single infusion (cohort 2a) or in 2 repeated infusions one week apart (cohort 2b). The dosage of Hepastem per infusion will be $0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion).

Each infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single infusion is expected to last minimum 10 min (for 10 mL of reconstituted Hepastem).

The full procedure describing how to adapat the volume of HepaStem to be administrated to the patient's body weight is in the HepaStem Manual.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate,ICUs or standard unitsdepending on the medical status of the patient and the organisation of study center hospital. Regardless of the unit of hospitalization, patients will remain hospitalised at least during the treatment period, with a close monitoring of each patient. During HepaStem infusion, a continuous monitoring of the vital signs of the patient is required.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a

specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension has to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation : the syringe has to be inversed 10 times every 3 minutes.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics,risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), and risks observed with the infusion of HepaStem in the cohort 1a, main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis or to consumption of coagulation factors and subsequent bleeding; respiratory disorder as cells first transit to the lungs; hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the risks mentioned below, there might be other, at this time, unknown risks.

5.4.1. Risk and Benefit assessment

ACLF patients or patients at high risk to develop ALCF are at high mortality risk and there is currently no specific treatment for these patients. Orthotopic liver transplantation is often not a possible option for these patients. By its potential combined effects, HepaStem could play a favourable role in restoring an

immunological balance in pre-ACLF / ACLF patients, leading to a resolution of this acute event and showing improvement of organ function and transplantation free survival. The main identified risks linked to HepaStem are activation of the coagulation cascade and may lead to thrombosis (observed in UCD patients) or bleeding (observed in ACLF patients). The safety measures described below (see section 5.5) are recommended to minimize the risks of the administration of HepaStem in ACLF or pre-ACLF patients at high risk of short term mortality.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the

Before each infusion, the investigator will have to make sure the patient has the minimum criteria to receive HepaStem (see 4.4 - Criteria for study treatment discontinuation).

Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lung disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect.

Number of cells administered for the cohort 1a, 2a and 2b per infusion will be maximum 25.10^6 cells (cohort 1a) or 50.10^6 cells (cohorts 2a and 2b) and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min. The lower dose regimen will be applied before the higher one. These doses are in the very low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication and re-adjusted to the range of MSC doses given in decompensated cirrhotic and ACLF patients (See Section 1).

Furthermore, **the coagulation parameters will be closely monitored** prior and after the infusion process at 4h, 8h, 12h, 24h, 48h and 72h post infusion. (Including INR, aPTT, fibrinogen, D-Dimers, coagulation factors (pre and 24h post infusion), and TEG (optional, only if measurement can be done locally and up to investigator's judgment)

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion, and patient with risk of bleeding (defined by recent major invasive procedure, non controlled gastrointestinal hemorrhage and/or coagulation disorders) will be excluded from the study.

In case major changes in the coagulation parameters and/or clinically significant bleedings suggestive of important coagulation factors consumption occur, according to the investigator's judgement, it could be envisioned to administer coagulation factors in the form of fresh frozen plasma (FFP), coagulation factor concentrate (ie Cofact containing Factors II, VII, IX, X plus protein S and protein C), fibrinogen concentrate (ie RiaSTAP), and/or antifibrinolytics (ie tranexamic acid). (cfr. both study patients in cohort 1a responded well to treatment with FFP and/or addition of coagulation factors).

In case of clinical signs or suspicion of pulmonary embolism, the investigator should perform exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigraphy.

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions. Any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement.

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Long Term Safety follow-up Period of 1 year..

Thereafter, patients will be invited to be followed-up Patient Registry (5 years).

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and treatment period, patients will be hospitalised in intermediate or ICUs or standard units, depending of the severity of the patient disease.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1

On HepaStem infusion days , before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, coagulation factors, TEG (if already performed as part of the clinical routine and up to investigator's judgment) a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

The careful evaluation of these parameters will allow or not the infusion (see section 4.4 – Criteria for treatment discontinuation).

During the infusion, the patient will be continuously monitored for early detection of any potential AEs.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

A study visit will be performed on Day 14 $\pm$ 2 days, including the evaluations listed below.

In case of any suspicion of AE, the investigator will perform the exams she/he evaluates as appropriate. In particular, any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement. In case of clinical signs or suspicion of pulmonary embolism, the investigator should perfom exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigrapy.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to the 28 days visit, all SAEs will be collected. After the 28 days visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of the transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be followed-up in the Patient Registry (5 years)

6.2.1. Study assessments

- All AEs up to Day 28
- All AESI up to 1 Year.
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation),MELD score and Child Pugh will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
    - GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
    - INR
    - aPTT
    - fibrinogen

- D-Dimers
- TEG (optional, only if measurement can be done locally and up to investigator's judgment)

- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion.
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening. Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2), 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also on M12
- Chest x-ray at screening (if not performed during same admission) and at M12,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)
- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

<u>Thromboelastogram and thromboglobulin generation tests</u>

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min twice). The plasma will be colleted and put in

cryotubes (min. 2 tubes containing each min. 500 µL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Hippocrate 10,
1200 Woluwe-Saint Lambert BELGIUM

Anti-HLA antibodies

Serum will be collected and send within 48 hours (ambient temperature) to :

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Hippocrate 10 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHARTS

Table 6-1 Study Flowchart

Period	**Screening Period**	**Active period**										
	Baseline	**Treatment Period**					**Surveillance Period**		***Long term follow-up***			
Time	**Over 1-4 days prior D1**	Infusion D1	D4[b]	D8[c]	D12[b]	D14[b]	**D21[b]**	**D28[b]**	**M2[d]**	**M3[d]**	**M6[d]**	**M12[e]**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	↔	↔	↔	↔	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	«[%]	«	«[%]	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«+	«	«+	«	X	X	X	X	X	X	X
Coagulation 2 : C-Protein, S-Protein, Anti-Thrombin III	X[§]											
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen, D-Dimers, TEG®		+		+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X[&]		X[&]								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	«		«		X		X				
TEG, TG	X	*	X	*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X	X	X				X
Chest X-Ray	©											X
Cardiac US Doppler	©	X										X
ECG	©											
Blood culture or other fluid culture	A											
~~Transjugular liver biopsy~~	~~O~~											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10[6] cells /kg body weight with a maximum of 25.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2a : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2b : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]		X[a]								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a) Hydrocortisone given 15-30 min before HepaStem infusion

b) ± 2 days

c) ± 2 days with at least 7 days interval without infusion

d) ± 2 weeks

e) ± 1 month

« Before each infusion .

A : If already performed during same admission, results collected

% : On infusion day, platelets measurement to be performed prior and post infusion at 4h, 24h, 48h and 72h.

© if not already performed during same admission; if already performed, results collected

ǂ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

≠ : cardiac US to be performed after infusion

@ : Optionnal, only if measurement can be done locally and up to investigator's judgment

+ : On infusion day : prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion (frequency of these exams can be increased up to the investigator's judgment.)

* : Before infusion and 4h post infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

& : Prior and 24h after infusion

§ : In case of deterioration of the coagulation, these measurements will be repeated.

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome

- Transplantation and outcome of the transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possible, probable or definite.

7.1.5. Serious Adverse Drug Reactions (SADR)

A SADR is any ADR that is serious as per the above criterias.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

<u>Possibly</u>

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probably

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definitly

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

Moderate, when interfering with ADL;

Severe, when impeding ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients

enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SADR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts each with 12 hospitalized patients in total.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

The 3 first patients infused (cohort 1a) : 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage (see section 1).

The next 3 patients cohort will receive a lower dose of HepaStem and the next 6 patients cohorts (high dose cohort) will receive the higher dose.

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Since it is a safety study, any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

The total sample size consideration remained unchanged with a total of 12 patients

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied.

Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of thetransplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], , if the patient is not able to fully understand the study based on the investigator's judgment, the informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is

initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline,

Promethera Biosciences must “verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection”.

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator’s Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient’s further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of external members and Promethera members including medical monitor, pharmacovigilance representative, clinical representive with expertise in liver disease or other relevant medical fields. The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.
2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.

As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort has received HepaStem infusions (complete scheme of premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

More specifically, based on the patients parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a unique administration of $0.5.10^6$ cells/kg body weight).

5. The SMC will review severe coagulation events assessed as related to HepaStem administration by the investigator.
6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- One or more biostatisticians
- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists may be invited to participate as non-voting members at any time if additional expertise is desired.

The frequency of SMC meetings will depend on several factors including the rate of enrollment, completion of patients in the dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL, renal replacement or use of vasopressors for Hepatorenal syndrome indication
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

Arroyo et al. 2015

11.2. CLIF ACLF GRADE

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

11.3. CLIF-C ACLF SCORE

CLIF-C ACLF = 10 × [(0,33 × CLIF OF + 0.04 × Age + 0,63 × Ln(WBC{10^9 cells/L}) – 2]

11.4. CLIF CONSORTIUM ACUTE DECOMPENSATION SCORE (CLIF-C AD)

CLIF-C AD = 10 × [(0,03 × Age {years} + 0,66 × Ln(Creatinine{mg/dL} + 1.71 × Ln(INR) + 0,88 × Ln(WBC{10^9 cells/L}) – 0,05 × Sodium {mmol/L} + 8]

Jalan et al. 2015

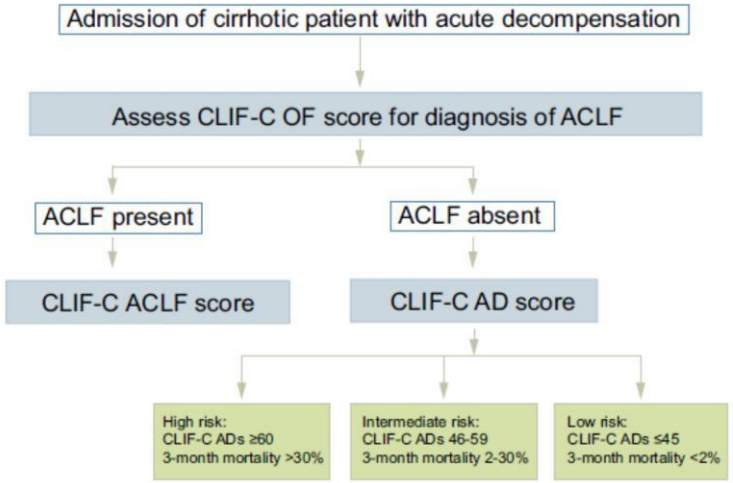

Fig. 4. Algorithm for the sequential use of the EASL-CLIF Consortium predictive scores in patients with cirrhosis admitted to hospital with acute decompensation.

11.5. MELD SCORE

MELD score is calculated using serum bilirubin, serum creatinine, and International Normalized Ratio (INR) and is given by the formula :

MELD(i) = (0.957 * ln(Serum Cr) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10 (if hemodialysis, value for Creatinine is automatically set to 4.0)

MELD Score (2016) = MELD(i) + 1.32*(137-Na) – [0.033*MELD(i)*(137-Na)]

Note: Sodium has a range of 125-137 mEq/L

11.6. THE SCORE CAN BE CALCULATED USING ONLINE WEBSITE HTTPS://WWW.MDCALC.COM/MELD-SCORE-MODEL-END-STAGE-LIVER-DISEASE-12-OLDERCHILD PUGH SCORE

Measure	1 point	2 points	3 points
Total bilirubin, μmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8
Prothrombin time prolongation (s)	<4.0	4.0–6.0	> 6.0

Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	Class	One year survival	Two year survival
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

Singal AK, Kamath PS. Model for End-stage Liver Disease. J Clin Exp Hepatol. 2013 Mar;3(1):50-60. Review.

11.7. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 21 Mar 2017

Version Number: 3.1

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____ _____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 21 Mar 2017

Version Number: 3.1

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competente Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

Silver Ocean Ventures SAS, CEO, represented by John Tchelingerian Promethera Biosciences	Date
Etienne Sokal, Chief Scientific & Innovation Officer Promethera Biosciences	Date
Nancy Veulemans, Vice-President Clinical & Medical Affairs Promethera Biosciences	Date
Decebal Bora, Vice-President Regulatory Affairs Promethera Biosciences	Date
Joelle Thonnard, Head of Medical Affairs and New Indications Promethera Biosciences	Date

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 3.2 – 11 May 2017

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Innovation & Scientific Officer John Tchelingerian, PhD – Chief Executive Officer Nancy Veulemans, Ir – Vice President Medical and Clinical Affairs Joëlle Thonnard, MD- Head of Medical Affairs and New Indication
MEDICAL MONITOR	Joëlle Thonnard, T.+32.471.563.423
MONITORING & STUDY COORDINATION	Virginie Barthel, T.+32.471.784.097
SAFETY & PHARMACOVIGILANCE	pv@promethera.com **T.*+32.10.39.43.43***

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AD	Acute Decompensation
ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SADR	Serious Adverse Drug Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 3.2.0 – 11 May 2017
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Twelve (12) evaluable Patients
Number of Centers	5-10 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total 5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next 3 patients enrolled will complete the first cohort and will receive a lower dose following SAEs observed in patient 2 and patient 3.

Six other patients will be enrolled in cohort 2.

The 3 first patients of cohort 2 (cohort 2a) will receive twice the dose compared to cohort 1b.

The 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

The statistical analysis will take into consideration the different doses applied.

Study periods

The study will recruit cirrhotic patients who are hospitalized for ACLF or Acute Decompensation at risk of developing ACLF

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Patients with Acute Decompensation of cirrhosis at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

The study is divided in the following periods: screening period, active study period divided in treatment period and evaluation period, and long-term safety follow-up.

Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days).The

duration of the screening period plus the active period will last up to 32 days (± 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Various dose regimens of HepaStem will be given, which differ in the amount of cells per infusion and/or in the number of infusions.

The 3 first patients received the dose regimen of 250.10^6 cells per infusion – this represents approximately $3.5.10^6$ cells/kg bodyweight in the first cohort. (cohort 1a)

The next three patients in cohort 1 (cohort 1b) will receive a lower dose (minimum ten times lower) in a single infusion ($0.25.10^6$ cells /kg bodyweight with a maximum of 25.10^6 cells per infusion).

The 3 next patients in cohort 2 (cohort 2a) will receive twice the dose of the patients in cohort 1b ($0.5.10^6$ cells/kg bodyweight with a maximum of 50.10^6 cells per infusion).

The 3 next patients in cohort 2 (cohort 2b) will receive up to 2 doses of $0.5.10^6$ cells/ kg bodyweight 1 week apart ($0.5.10^6$ cells/kg bodyweight per infusion with a maximum of 50.10^6 cells per infusion).

Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of members, all external and independent to Promethera Biosciences.

As a minimum, the safety data will be reviewed by the SMC at the following timepoints :

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received the HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advice on the enrolment of patients in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patients' parameters in cohort 2a, the SMC

	might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg bodyweight) instead of a single administration of $0.5.10^6$ cells/kg bodyweight). Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period. After completion of this study, patients will be followed-up in the Patient Registry.
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and thereafter for an additional period of 1 year in the long term safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at $50x10^6$ cells/ml equivalent to $250x10^6$ cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent. In cohort 1a, 50 ml was given per infusion. For cohorts 1b, 2a and 2b, the volume of HepaStem administered will be adapted to the patient's bodyweight.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on the patient's status. The infusion rate shall not exceed 1 ml per min. For cohort 1, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion had to be respected between infusion days. The *Planned* schedule was : in cohort 1a, 250 million cells in 50 ml were administered on each infusion day, leading to a total of 1 billion cells ,if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone was expected to be given 15 to 30 min before each HepaStem infusion. The *Actual* schedule is: in cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to

	30 min before each HepaStem infusion. For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2a: 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).
Eligibility - Inclusion Criteria	<u>Inclusion criteria</u>: 1. Adult aged between 18 and 70 year old. 2. Informed Consent. N.B: In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement. 3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan). 4. Patient with Acute Decompensation of cirrhosis 5. Serum total Bilirubin ≥ 6 mg/dL (≥100 umol/L) 6 The INR measurement has to be : 1.2 ≤ INR < 2

Eligibility - Exclusion Criteria	Exclusion criteria: 1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam. 2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion. 3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion. 4. Variceal banding or sclerosis within 4 weeks before the infusion. 5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 6. Clinical evidence of Aspergillus infection. 7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion. 8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation. 9. Coagulation disorders defined as : • INR ≥ 2 • Fibrinogen < 100 mg/dL • Platelets < 50.000/mm^3 10. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator. 11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period. 12. MELD score > 30. 13. Previous organ transplantation and/or ongoing immunosuppressive treatments. 14. Postoperative-decompensation following hepatectomy. 15. Renal failure due to chronic kidney disease. 16. Clinically significant left-right cardiac shunt. 17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or

	foods or a history of severe anaphylactic reactions. 18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. 19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 21. Participation to any other interventional study within the last 4 weeks. 22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisousness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28. The relationship will be assessed based on investigator assessment, and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline. <u>Secondary Endpoints</u>: • Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Mortality ○ Liver transplantation ○ Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score, Child Pugh score. • Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Bilirubin, creatinine, INR and albumin values • Long term safety follow-up ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1 ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation New

	ACLF episode will be summarized at Month 3 and Year 1
Study Assessment visits	Study visits During the screening and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. On HepaStem infusion days,, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, TEG (if already performed as part of the clinical routine and up to investigator's judgment), coagulation factors (intrinsic and extrincsic pathway), a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day (even if HepaStem infusions are prematurily stopped). The careful evaluation of these parameters will allow or will not allow the infusion (see section 4.4 – Criteria for treatment discontinuation). On the other days during the hospital stay, patients will be followed-up according to usual practice. A study visit will be performed on Day 4, 8, 12 and 14 ± 2 days post 1st infusion, including the evaluations listed below. After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged. After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month). Up to Day 28 visit, all SAEs will be collected. After Day 28 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including

	SAE with fatal outcome, liver transplantation, malignancies, new AD and/or ACLF episode, AEs assessed by the investigator as possibly related to HepaStem (see Section 7.1.2).
	
	At Month 12 study visit, patients will be invited to be included in the Patient Registry.
	
	<u>Study assessments</u>
	
	• All AEs up to Day 28
	• All AESI up to 1 Year.
	• Concomitant medication modifications up to Day 28
	• Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD score (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12,, 14, 21, 28, Months 2, 3. 6 and 12
	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
	  ○ GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
	  ○ Creatinine, Urea or BUN
	  ○ CRP
	  ○ INR, aPTT
	  ○ Serum albumin, sodium, potassium
	• Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
	  ○ INR
	  ○ aPTT
	  ○ fibrinogen

	○ D-Dimers ○ TEG (optional, only if measurement can be done locally and up to investigator's judgment) • Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion. • Lipase: at screening • Viral serology (HIV, HCV, HEV, HbS antigen) and Aspergilus detection: at screening (if not performed during same admission) • Urine test (Sediment, Creat, Glc, Protein, Albm): at screening • Protein C, Protein S, anti-thrombin III: at screening. In case of deterioration of the coagulation, these measurements will be repeated. • Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2b), 12, 14, 21, 28 (blood testing in central lab) • Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab) • Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab) • Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also at M12. • Chest x-ray : at screening (if not performed during same admission) and at M12, • Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected) • ECG: at screening (if not performed during same admission). • Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12. A SMC will review safety data and advise on study conduct. Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion. In case of premature withdrawal from study, an end of study visit should be perfomed if possible at the time of study withdrawal. In case of liver transplantation during the course of the study, a sample of the explanted liver will be collected if possible.

Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem is administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist. The 3 first patients infused (cohort 1a) received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. 2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage to a new low dose. (see section 1)). The next 3 patients will receive a lower dose of HepaStem and the next 6 patients (new high dose cohort) will receive the higher dose. The total sample size consideration remains unchanged with a total of 12 patients.
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who received at least one infusion.

	All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after treated patients of cohorts 1 and 2

	will have completed the 28 day active study period or have died or have been lost to follow-up. The Report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Period	**Screening Period**	**Active period**							*Long term follow-up*			
	Baseline	**Treatment Period**					**Surveillance Period**					
Time	**Over 1-4 days prior D1**	Infusion D1	D4[b]	D8[c]	D12[b]	D14[b]	**D21[b]**	**D28[b]**	**M2[d]**	**M3[d]**	**M6[d]**	**M12[e]**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	↔	↔	↔	↔	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	«%	«	«%	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«+	«	«+	«	X	X	X	X	X	X	X
Coagulation 2 : C-Protein, S-Protein, Anti-Thrombin III	X§											
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen, D-Dimers, TEG@		+		+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	«		«		X		X				
TEG, TG	X	*	X	*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X	X	X				X
Chest X-Ray	©											X
Cardiac US Doppler	©	X										X
ECG	©											
Blood culture or other fluid culture	A											
~~Transjugular liver biopsy~~	~~O~~											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶ cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg)		X[a]										
Cohort 2a : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg)		X[a]										
Cohort 2b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg)		X[a]		X[a]								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a) Hydrocortisone given 15-30 min before HepaStem infusion

b) ± 2 days
c) ± 2 days with at least 7 days interval without infusion
d) ± 2 weeks
e) ± 1 month
« Before each infusion .
A : If already performed during same admission, results collected
% : On infusion day, platelets measurement to be performed prior and post infusion at 4h, 24h, 48h and 72h.

© if not already performed during same admission; if already performed, results collected

ǂ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)
≠ : cardiac US to be performed after infusion
@ : Optionnal, only if measurement can be done locally and up to investigator's judgment
+ : On infusion day : prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion (frequency of these exams can be increased up to the investigator's judgment.)

* : Before infusion and 4h post infusion
AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation
& : Prior and 24h after infusion

§ : In case of deterioration of the coagulation, these measurements will be repeated.

1. BACKGROUND AND RATIONALE

1.1. CIRRHOSIS, ACUTE DECOMPENSATION AND ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Cirrhosis is a progressive chronic liver disease characterized by diffuse fibrosis, severe disruption of the intrahepatic venous flow, portal hypertension and liver failure. The course of cirrhosis is divided into two stages. Compensated cirrhosis defines the period between the onset of cirrhosis and the first major complication. During this period, which is relatively long in most patients (>10 years), symptoms are absent or minor, but liver lesions and portal pressure steadily progress. The term decompensated cirrhosis defines the period following the development of ascites (that is, the accumulation of large amounts of fluid within the peritoneal cavity), variceal haemorrhage and/or hepatic encephalopathy. This period is associated with short-term survival (3–5 years). It is increasingly evident that patients rarely die as a consequence of an end-stage irreversible destruction of the liver. Rather, in most patients, the cause of death is an acute deterioration in their chronic clinical condition promoted by a precipitating event — a syndrome termed acute-on-chronic liver failure (ACLF) (Arroyo et al. 2015).

It is of note that definitions on ACLF may differ worldwide. Given the heterogeneity and the importance of identifying patients four major societies/organisations have provided working definitions (APASL, NACSELD, WGO and EASL-CLIF). The common definition of ACLF is 'a syndrome characterised by acute decompensation of chronic liver disease associated with organ failure(s) and high short-term mortality'. According to the CLIF-ACLF definition developed based on the CANONIC study, ACLF is a recognised syndrome characterised by acute decompensation of cirrhosis associated with the failure of one or more organs and, in the more severe cases, system failure. The organs and systems most likely to fail are the liver, kidney, brain, coagulation, circulation and/or lungs. Patients have a high short term mortality of over 15 % at 28 days (Hernaez R et al, 2017). In the CANONIC study approximately 31% of patients admitted to a hospital for Acute Decompensation (AD) of cirrhosis had ACLF at admission (20%) or developed the syndrome during hospitalisation (11%). The common causes of acute decompensation of liver function included bacterial infections, alcoholic hepatitis, and gastrointestinal hemorrhages, but, in more than 40 % of patients, no precipitating event was identified (Moreau et al. 2013). Among patients with Acute Decompensation (AD), subgroups were identified as being at higher risk of progressing to full blown ACLF and thus at higher mortality risk (Arroyo et al. 2015).
Different grading/scoring systems have been developed in order to better determine prognosis and effectiveness of intervention and care. (Hernaez R et al, 2017).

In daily practice, MELD and Child Pugh scores are still strongly relied on to guide clinical care.

The Model for End-Stage Liver Disease, or MELD, is a scoring system for assessing the severity of chronic liver disease. This score is used by the United Network for Organ Sharing (UNOS) and Eurotransplant for prioritizing allocation of liver transplants. New MELD uses the patient's values for serum bilirubin, serum creatinine, sodium and the international normalized ratio for prothrombin time (INR) to predict survival.

Mortality and MELD score are linearly correlated amongst patients with end-stage liver disease listed for OLT with 3-month mortality estimated to be 4%, 27%, 76%, 83%, and 100% for MELD scores of <10, 10–19, 20–29, 30–39, and 40 or more respectively.

The Child–Pugh score is used in clinical practice to assess the prognosis of chronic liver disease, mainly cirrhosis. It was previously used for prioritizing allocation of liver transplants. The score employs five clinical measures of liver disease: total bilirubin, serum albumin, prothrombin time, ascites and hepatic encephalopathy. Each measure is scored 1–3, with 3 indicating most severe derangement. This leads to three Classes with one year overal survival of 100% for Class A, 81% for class B and 35% for class C. (see 0)

ACLF has been defined by the CLIF research consortium into four grades based on retrospectively fitting data on severity linked to mortality score (Moreau et al. 2013) (Table 1-1 and Table 1-2)

- ACLF grade 0 concerns 69.1 % of patients admitted to hospital with acute decompensation. The group is defined as no organ failure, single "non kidney" organ failure (ie, single failure of the liver, coagulation, circulation, or respiration) who had a serum creatinine level < 1.5 mg/dL and no hepatic encephalopathy, or as single cerebral failure with a serum creatinine level < 1.5 mg/dL. These patients have a 28-day and 90-day mortality rate of 4.7% and 14% respectively.
- ACLF grade 1 concerns 15.8 % of patients admitted to hospital with acute decompensation. The group is defined as single kidney failure or single non-kidney organ failure with an organ dysfunction (kidney or brain) and has a 28-day mortality rate of 23 %.
- Patients with ACLF grade 2, defined as two failing organs (10.9 % of patients admitted to hospital with acute decompensation) has an intermediate prognosis (28-day mortality rate of 31%).
- Finally, ACLF grade 3, defined as three or more organ failures (4.4 % of patients admitted to hospital with acute decompensation) has extremely high mortality rates, reaching 75 % after 28 days.

Among patients hospitalised with acute decompensation (AD) (pre ACLF according to the CLIF criteria but ACLF according to other classification systems), an analysis revealed five independent variables including age, serum sodium, white cell count, creatinine and INR as useful for defining a scoring system. The high-risk group (CLIF-C AD score > 60) and intermediate risk group (CLIF-C AD score 46-59) respectively have a 3-month mortality of over 30 % and between 2-30 %. The low risk AD group has a 3-month mortality below 2 % (Arroyo et al. 2015).

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years.The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

Cirrhotic patients with acute decompensation can only receive supportive treatments, such as antibiotics in case of infection, lactulose in case of encephalopathy, terlipressin and albumin in case of hepatorenal syndrome. However, at this moment, there are no treatments available to stop the inflammatory cascade often accompanying the acute decompensation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion,

which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 , renal replacement or use of vasopressors for Hepatorenal syndrome indication
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may cause necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver transplant rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

Conclusion on patient population: Based on this information, Promethera Biosciences proposes that the patient population is defined as cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 µmol/L) and MELD < or = 30.

Patients should have coagulation parameters within the ranges below:

- INR ≥ 1.2 and < 2
- Fibrinogen ≥ 100 mg/ dL
- Platelets ≥ 50.000/mm3

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidence supports the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properties of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells (similar cells to HHALPC) were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013).

Later, the technology of large-scale cell production was transferred to Promethera Biosciences where clinical batches of HHALPC are produced in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs).

Toxicology *in-vitro or in-vivo* studies aiming to demonstrate the safety, tolerability and tumorigenicity aspect of HepaStem were conducted. *In vivo* studies were performed in rats and mice. They included one study to assess the safety of the intravenous mode of administration. Two studies specifically assessed the risk of tumor formation as this risk has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. An *in vitro* study the pro-coagulant activity of HepaStem was confirmed (Please refer to the IB for more details).

In addition, *invitro* studies show that HepaStem cells express variable immunomodulatory surface markers of interest and have immunomodulatory functional effects: HepaStem inhibits the proliferation of activated T-lymphocytes and blocks the maturation of monocytes (see Section 1.2.6). Furthermore, 6 *in-vivo* studies were conducted with HepaStem evaluating the immunomodulatory properties using the IV route of administration and mainly doses of 12.5 10^6 cells/kg. No safety signal was detected based on these *in vivo* studies.

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg) (4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few

days. None did undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. These data laid the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

Based on literature data and Promethera experiences, it can be concluded that liver-derived MSCs, including HepaStem have a pro-coagulant activity. This pro-coagulant activity is also expressed by other MSCs. The pro-coagulant activity might be linked to tissue factor expression, an activator of the coagulation cascade. The procoagulant effect could be modulated by the concomitant administration of bivaluridin during HepaStem infusion in UCD clinical trials in order to prevent, mainly, anticipated thrombotic events. Very high cell doses have been administered intra-portally in the UCD studies in which thrombotic events only occurred at high doses (range: 115 million to 4,1 billion total cells were administered in the portal vein as a split dose in 1 to 10 infusions spread over 1 to 4 consecutive days). Bivaluridin will not be used in the ACLF clinical study as its use has not been validated for late stage cirrhotic patients. Contrary to patients with urea-cycle disorders, coagulation disturbances are common in the late stage chronic cirrhosis population and are linked to liver insufficiency. (see 1.2.5)

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

In a first-in man cohort conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells (ADHLSC, similar cells to HHALPC) infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated this cell infusion well, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6

cells repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, spleen, right ankle, and spine, and had disappeared from the lungs. This is in line with the bio-distribution of another type of MSCs administrated in patients (BM-derived MSCs) that demonstrate a similar bio-distribution, with a first pass through the lung; within 24 hours, cells are mainly found in liver, spleen, kidneys and other inflamed areas, by 48 hours, more pronounced presence in the liver is observed. (NDS dossier remestemcel-L, Health Canada).

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For example, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012).

In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

It is clear from consultation of literature publications on cell therapy administration in advanced liver disease including decompensated liver cirrhosis and ACLF that doses of cell therapy protocols tended to be lower as compared to the normal range administered to patients in other immune-modulatory or anti-inflammatory protocols.

The doses and regimens administered to treat patients with chronic liver diseases, range from 0.03 over 0.5 to 1 million MSC cells/kg bodyweight. Different regimens were applied with repeated dosing up to 3 times for the lowest doses (0.03; 0,05 and 0,5 million cells/kg BW repeated 3 times). Most protocols administered the cell infusion intravenously although also other routes of administration were investigated such as intra-splenic, hepatic artery, intrahepatic, intra-lesional route of administration or central venous catheter into the femoral vein. (Berardis et al. 2015)

Based on the literature review, MSC administration is considered to be safe due to the lack of reports of significant adverse effects in the above studies, although a marked heterogeneity was observed among studies with regard to injection dose, frequency of injection, cell source, delivery route and study design. Most of these early studies reported improvements in liver function, ascites and encephalopathy.

In the first cohort of 3 patients in the HEP001 study the lowest dose (12.5×10^6 cells/kg) of the range of doses administered safely in previous studies of HepaStem in urea cycle disorder (UCD) and Crigler Najar pediatric patients was used to determine the dose in the HEP101 protocol. The (low) dose proposed (250

million cells/ infusion; ie. 3.5 10^6 cells/kg BW/infusion) was a reduction of 4x of the lowest dose tested previously (in the HEP001 protocol) and it was thought that it could be safely administered in cirrhotic patients. Additionally, the number of cells administered per infusion would be limited, similar to MSC doses given in immune mediated inflammatory diseases. In retrospect, it was clear that adaptation to the dose level similar to other MSCs given in immune-mediated inflammatory diseases was inadequate and did not take the specific case of severely ill chronic cirrhotic patients with acute decompensation into account.

Therefore, it seems that using a careful approach starting with doses commonly used in reported studies of decompensated cirrhosis and ACLF patients and published as being safe, appears to be an acceptable approach. Also, dose escalation to a maximum of 1.0 million cells/kg BW should be feasible based on a repetitive dosing schedule. In case of repetitive dosing, the doses will be given weekly, which allows time in case of fibrinolysis for the parameters to be corrected and return to normal. (please refer to rationale for changes)

1.2.6. Pre-clinical immunomodulatory data of liver-derived progenitor cells

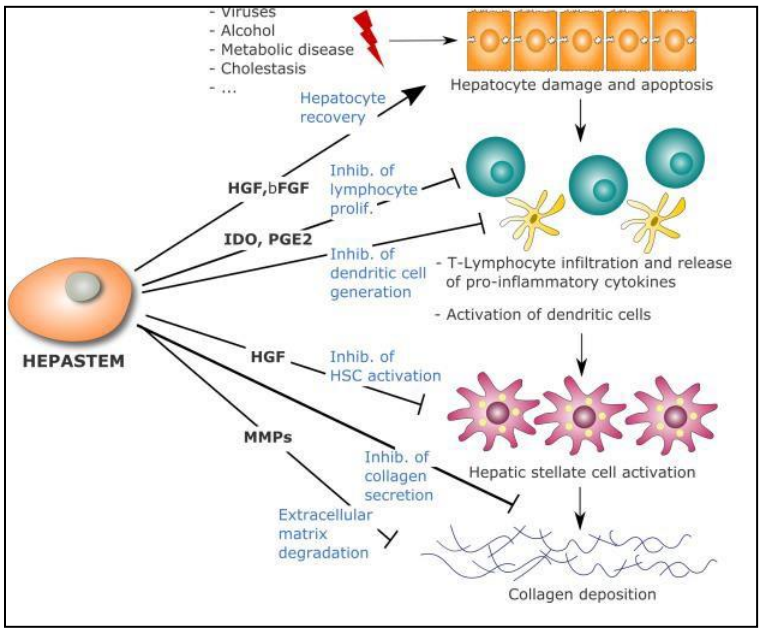
The first transcriptomics and secretomics tests performed on liver-derived progenitor cells (similar cells to HHALPC) grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (similar cells to HHALPC) (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of

HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models presents important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.7. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing to the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed

in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.8. Expected Benefits of HepaStem

Proposed mechanisms of action: after intravenous administration of HepaStem, cells are expected to circulate into the blood network where they can exert a systemic immunomodulatory action. At the same time, they have a main homing into the liver where they can thus also exert some important local immunomodulatory effects. They are expected to play their immunomodulatory roles through direct cell-to-cell interaction and through paracrine effects via the various cytokines, chemokines, MMPs and growth factors they may secrete. HepaStem could affect monocytes and DC recruitment, differentiation, maturation and function through cell contacts or paracrine signalling. HepaStem could also alter the proliferation and activation of T-lymphocytes that are another dysregulated cell type of the immune system in ACLF. In addition to modulate the behaviour of immune cells, HepaStem could modulate the proliferation and activation of hepatocytes and hepatic stellate cells and thus their secretory profiles, helping in this way the liver function recovery. The current *in vitro* and *in vivo* data, based on the scientific literature, and sponsor *in vitro* results, support all these potential immunomodulatory effects of HepaStem in ACLF patients.

Proposed clinical significant benefit: by these combined effects, HepaStem could play a favourable role in restoring an immunological balance in ACLF patients or patients at risk of ACLF, improving organ failure scores, improving clinical status, possibly leading to a resolution of this acute event and demonstrating improvement of transplantation free survival.

Considering the unmet medical need: i. the emergency to treat cirrhotic patients with Acute Decompensation (pre-ACLF or ACLF) due to the high mortality rate; ii. the shortage of healthy donors and the need of livers in the context of liver transplantation; iii. Concerns raised recently regarding artificial liver support; and iv. the mechanism of action of HepaStem, we can say that all these factors are in favour of a promising favourable benefit/risk balance for HepaStem. The exact profile of which patients will benefit most is under investigation, and also subject of this safety study.

1.2.9. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total. The study will include patients with an acute decompensation of cirrhosis and with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 30,excluding patients with circulatory,respiratory failure or severe coagulations disorders). It is planned to have a first group of 6 patients (cohort 1) being administered with the low dose.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between

infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 patients experienced an episode of severe bleeding. Therefore, it has been decided to reduce the dose in the low dose cohort to $0.25.10^6$ cells/kg bodyweight with a maximum of 25.10^6 cells in a single infusion. A reduction of minimum 10 times the dose previously used.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

Once this has been proven safe, a second group of 3 patients (cohort 2a) will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 6 patients, appears to be an acceptable approach in patients with ACLF or with acute decompensation at risk of developing ACLF for whom no specific therapeutic or curative treatment exist. (See section 1.1)

HepaStem administration will be started rapidly after hospitalisation and will be completed within 1 day (cohort 1b) or within 1 week (cohort 2b). This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of patients with or at risk of developing ACLF. As ACLF and/or Acute Decompensation of cirrhosis is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction

or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up for safety up 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining the design of future efficacy clinical studies.

Study population

The patient population is defined by cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 30 (see section 1.1).

Patients should have coagulation parameters within the ranges below:

- INR ≥ 1.2 and < 2
- Fibrinogen ≥ 100 mg/ dL
- Platelets ≥ 50.000/mm3

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 14 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 28 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 million cells per infusion) is close to MSC doses given in other trials for immune-mediated inflammatory diseases (Section 1.2.5), therefore, it was expected to show a similar safety and efficacy profile. It corresponded also to the high dose of liver-dirived progenitor cells (similar cells to HHALPC) administered via IV to the heamophila patient (see 1.2.4).

Due to the severe bleeding that occurred in 2 of the 3 patients that received 250 10^6 cells (50 mL of HepaStem) per infusion, the next selected dose (low dose cohort 1b) will be reduced to 0.25.10^6cells/kg bodyweight (with a maximum of 25.10^6 cells per infusion) administered in a single infusion (at least a 10x reduction of the dose administered in cohort 1a).

The second selected dose represents a two-fold increase from the dose in cohort 1b (0.5.10^6cells/kg bodyweight (with a maximum of 50.10^6 cells per infusion) still in the range of doses reported for MSCs and more in the range of doses administered in the specific case of severely ill chronic liver disease

patients with ACLF and acute decompensation of cirrhosis. (see1.2.5)). (for additional information, please refer to the rationale for changes).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 IU/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damage nor any respiratory symptoms, before homing mainly to the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF , it also can allow repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs also have a procoagulant activity comparable to liver-derived progenitor cells (similar cells to HHALPC) (Stephenne et al. 2012), nevertheless literature reports show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015).

However, liver failure results in a state of “rebalanced hemostasis” marked by a decrease in both pro-coagulation and anticoagulation factors. Patients with severe liver disease are not auto-anticoagulated. In essence, patients with severe liver disease, acute and/or chronic, have a tenuous rebalanced hemostasis that is easily perturbed by various disease states and concomitant medications and invasive procedures. Bleeding events including severe forms are common in these end-stage liver disease patients. The events of epistaxis and bleeding from puncture sites that occurred in 2 patients in cohort 1a (in retrospect a high dose in late stage cirrhotic patients), have been recognised in the literature as case reports. It was also stated that epistaxis as an overlooked cause of massive haematemesis in cirrhosis should be added to the list of upper GI bleeding.(Johal et al 2003). Hence, cirrhotic patients including ACLF patients are at increased risk of bleeding or thrombosis. Therefore dose reduction from normal ranges applied in other immune-modulatory diseases, modification of inclusion criteria and increased surveillance of liver and coagualation parameters is indicated.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of different regimens of HepaStem in cirrhotic patients presenting with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total.

5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next 3 patients enrolled will complete the first cohort and will receive a lower dose following SAEs observed in patient 2 and patient 3.

Six other patients will be enrolled in cohort 2.

The 3 first patients of the cohort 2 (cohort 2a) will receive twice the dose compared to the cohort 1b.

The 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

The study will recruit patients who are hospitalized for Acute Decompensation of cirrhosis and/or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

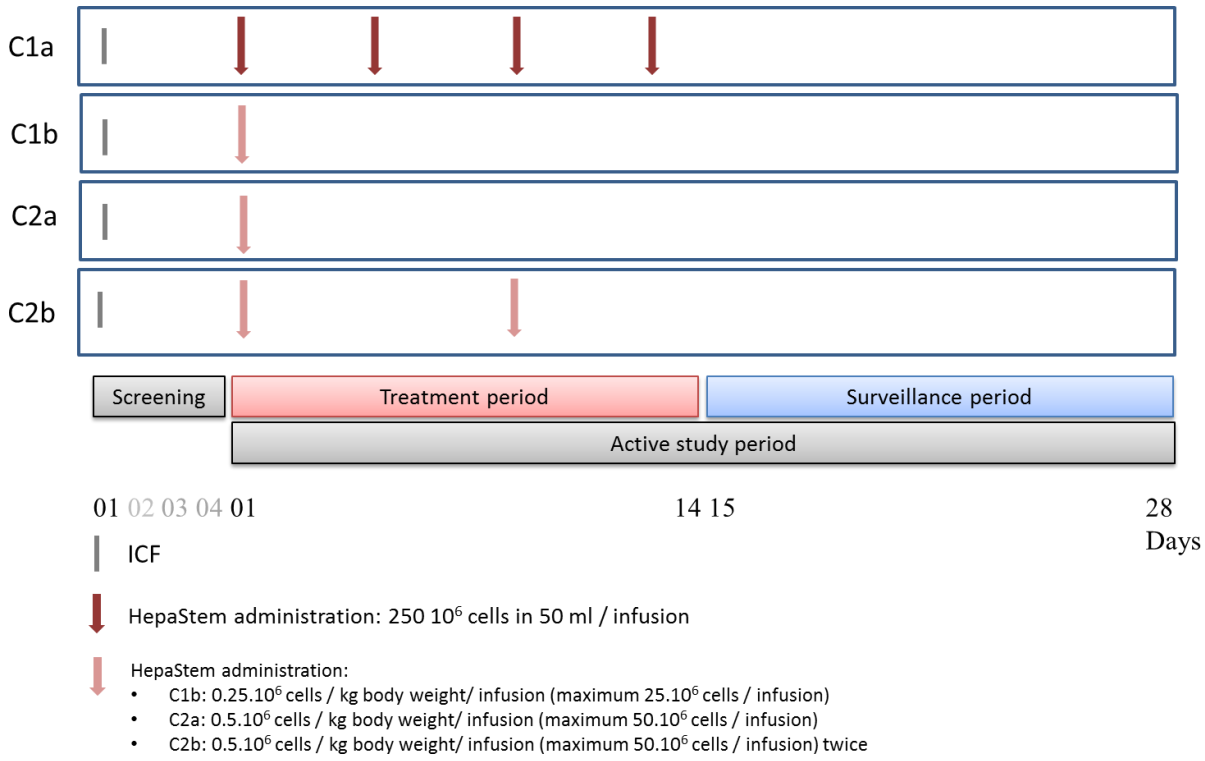
Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days ($\pm$ 2 days). The duration of the screening period plus the active period will last up to 32 days ($\pm$ 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



Planned schedule:

For cohort 1a, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion must be respected between infusion days.

In cohort 1a, 250 million cells in 50 ml were administrated on each infusion day, leading to a total of 1 billion cells if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion.

Actual schedule:

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

Planned schedule:

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

For cohort 2a: 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of external members and Promethera members (See Section 9.13):

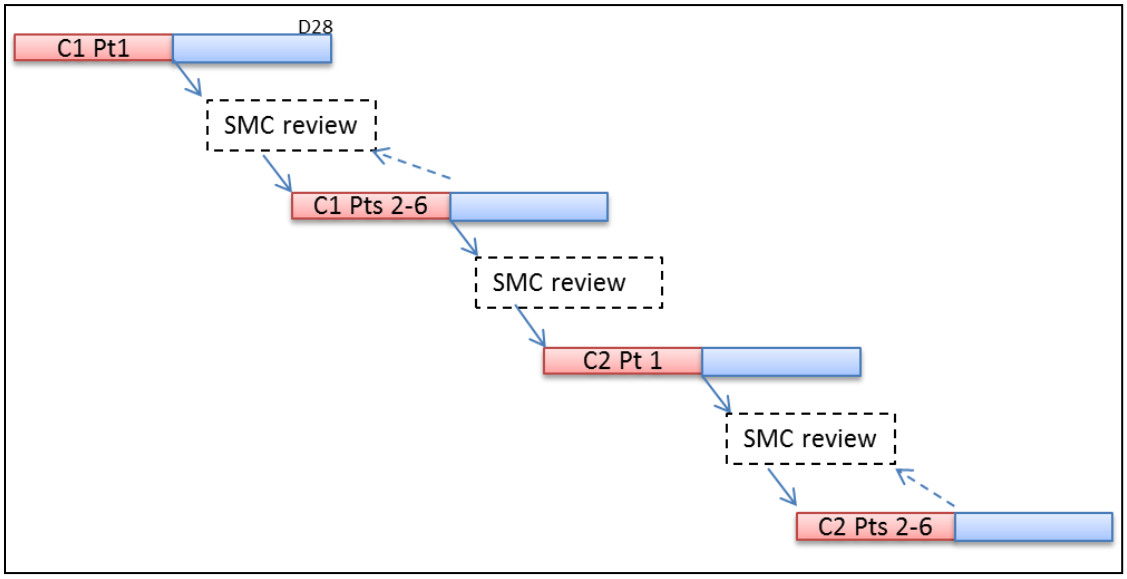
As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patients parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a single administration of $0.5.10^6$ cells/kg body weight).

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring Committe



The study assessments are described in Section 6.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be followed-up in the Patient Registry.

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
    - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation
- New ACLF episode will be sumamrized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in 5 to 10 clinical centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days ($\pm$ 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Cirrhotic patients with Acute Decompensation at risk of developing ACLF at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
   N.B: In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.
3. Cirrhosis as diagnosed by
   - liver histology or
   - clinical and imaging examination (may include fibroscan).
4. Patient with Acute Decompensation of cirrhosis
5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)
6. The INR measurement has to be : $1.2 \leq INR < 2$

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.

   Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.

3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion.
4. Variceal banding or sclerosis within 4 weeks before the infusion
5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
6. Clinical evidence of Aspergilus infection.
7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion.
8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
9. Coagulation disorders defined as :
    - INR ≥ 2
    - Fibrinogen < 100 mg/dL
    - Platelets < 50.000/mm^3
10. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator.
11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.
12. MELD score > 30.
13. Previous organ transplantation and/or ongoing immunosuppressive treatments.
14. Postoperative-decompensation following hepatectomy.
15. Renal failure due to chronic kidney disease.
16. Clinically significant left-right cardiac shunt.
17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
21. Participation to any other interventional study within the last 4 weeks.
22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration are the following:

- **Transitory discontinuation:** Coagulation disorders considered as significant (INR ≥ 2, Fibrinogen < 100 mg/dL, or Platelets < 50.000/mm^3) by the PI prior to each infusion should preclude the administration of Hepastem.
- Absence of portal vein flow prior to the infusion should preclude the administration of Hepastem.
- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.

Infusions may be restarted after recovery. If planned infusions are not performed within treatment period (± 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAWAL CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be

reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).
- The exact dosage (volume) of Hepastem infused to the patient will be calculated based on the weight of the patient on the day of infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells/infusion (5 mL) for cohort 1b or $0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells/infusion (10 mL) for cohort 2.)
- As the exact volume to infused can be low (depending on the patient's weight), it is recommended to flush after the infusion physiological solution (NaCl 0,9%) to ensure that all the product is infused.

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the

mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and the syringe will be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells per infusion). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapated accordingly. A single infusion is expected to last minimum 5 min (for 5 mL of reconstituted Hepastem).

For cohort 2a and 2b: 3 patients will receive HepaStem in a single infusion (cohort 2a) or in 2 repeated infusions one week apart (cohort 2b). The dosage of Hepastem per infusion will be $0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion).

Each infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single infusion is expected to last minimum 10 min (for 10 mL of reconstituted Hepastem).

The full procedure describing how to adapat the volume of HepaStem to be administrated to the patient's body weight is in the HepaStem Manual.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate,ICUs or standard units. Patients will be hospitalised during HepaStem treatment period to allow a continuous monitoring of the patient.

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension has to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation : the syringe has to be inversed 10 times every 3 minutes.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics,risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), and risks observed with the infusion of HepaStem in the cohort 1a, main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis or to consumption of coagulation factors and subsequent bleeding; respiratory disorder as cells first transit to the lungs; hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the risks mentioned below, there might be other, at this time, unknown risks.

5.4.1. Risk and Benefit assessment

ACLF patients or patients at high risk to develop ALCF are at high mortality risk and there is currently no specific treatment for these patients. Orthotopic liver transplantation is often not a possible option for these patients. By its potential combined effects, HepaStem could play a favourable role in restoring an immunological balance in pre-ACLF / ACLF patients, leading to a resolution of this acute event and showing improvement of organ function and transplantation free survival. The main identified risks linked to HepaStem are activation of the coagulation cascade and may lead to thrombosis (observed in UCD patients) or bleeding (observed in ACLF patients). The safety measures described below (see section 5.5) are recommended to minimize the risks of the administration of HepaStem in ACLF or pre-ACLF patients at high risk of short term mortality.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the

Before each infusion, the investigator will have to make sure the patient has the minimum criteria to receive HepaStem (see 4.4 - Criteria for study treatment discontinuation).

Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lung disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect.

Number of cells administered for the cohort 1a, 2a and 2b per infusion will be maximum 25.10^6 cells (cohort 1a) or 50.10^6 cells (cohorts 2a and 2b) and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min. The lower dose regimen will be applied before the higher one. These doses are in the very low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication and re-adjusted to the range of MSC doses given in decompensated cirrhotic and ACLF patients (See Section 1).

Furthermore, **the coagulation parameters will be closely monitored** prior and after the infusion process at 4h, 8h, 12h, 24h, 48h and 72h post infusion. (Including INR, aPTT, fibrinogen, D-Dimers, coagulation factors (pre and 24h post infusion), and TEG (optional, only if measurement can be done locally and up to investigator's judgment)

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion, and patient with risk of bleeding (defined by recent major invasive procedure, non controlled gastrointestinal hemorrhage and/or coagulation disorders) will be excluded from the study.

In case major changes in the coagulation parameters and/or clinically significant bleedings suggestive of important coagulation factors consumption occur, according to the investigator's judgement, it could be envisioned to administer coagulation factors in the form of fresh frozen plasma (FFP), coagulation factor concentrate (ie Cofact containing Factors II, VII, IX, X plus protein S and protein C), fibrinogen concentrate (ie RiaSTAP), and/or antifibrinolytics (ie tranexamic acid). (cfr. both study patients in cohort 1a responded well to treatment with FFP and/or addition of coagulation factors).

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions.

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Long Term Safety follow-up Period of 1 year. Thereafter, they will be followed-up the the Patient Registry.

Thereafter, patients will be followed in the Patient Registry.

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1

On HepaStem infusion days, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, coagulation factors, TEG (if already performed as part of the clinical routine and up to investigator's judgment) a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

The careful evaluation of these parameters will allow or not the infusion (see section 4.4 – Criteria for treatment discontinuation).

On the other days during the hospital stay, patients will be followed-up according to usual practice.

A study visit will be performed on Day 14 $\pm$ 2 days, including the evaluations listed below.

After the treatment period, study visits will be done on days 21 and 28 ($\pm$ 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 ($\pm$ 2 weeks), Month 3 ($\pm$ 2 weeks), Month 6 ($\pm$ 2 weeks), and Year 1 ($\pm$ 1 month).

Up to the 28 days visit, all SAEs will be collected. After the 28 days visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be included in the patient Registry.

6.2.1. Study assessments

- All AEs up to Day 28

- All AESI up to 1 Year.
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation),MELD score and Child Pugh will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
    - GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
    - INR
    - aPTT
    - fibrinogen
    - D-Dimers
    - TEG (optional, only if measurement can be done locally and up to investigator's judgment)
- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion.
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening. Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2), 12, 14, 21, 28 (blood testing in central lab)

- Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also on M12
- Chest x-ray at screening (if not performed during same admission) and at M12,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)
- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

<u>Thromboelastogram and thromboglobulin generation tests</u>

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min twice). The plasma will be colleted and put in cryotubes (min. 2 tubes containing each min. 500 µL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Hippocrate 10,
1200 Woluwe-Saint Lambert BELGIUM

<u>Anti-HLA antibodies</u>

Serum will be collected and send within 48 hours (ambient temperature) to :

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Hippocrate 10 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHARTS

Table 6-1 Study Flowchart

Period	**Screening Period**	**Active period**							*Long term follow-up*			
	Baseline	**Treatment Period**					**Surveillance Period**					
Time	**Over 1-4 days prior D1**	Infusion D1	D4[b]	D8[c]	D12[b]	D14[b]	**D21[b]**	**D28[b]**	**M2[d]**	**M3[d]**	**M6[d]**	**M12[e]**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	«	«	«	X	X	X	X	X	X	X
Vital Sign	X	↔	↔	↔	↔	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	«[%]	«	«[%]	«	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	«	«	«	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	«+	«	«+	«	X	X	X	X	X	X	X
Coagulation 2 : C-Protein, S-Protein, Anti-Thrombin III	X[§]											
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen, D-Dimers, TEG@		+		+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X[&]		X[&]								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	«		«		X		X				
TEG, TG	X	*	X	*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	«	«	«	X	X	X				X
Chest X-Ray	©											X
Cardiac US Doppler	©	X										X
ECG	©											
Blood culture or other fluid culture	A											
~~Transjugular liver biopsy~~	~~O~~											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10[6] cells /kg body weight with a maximum of 25.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2a : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2b : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]		X[a]								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a) Hydrocortisone given 15-30 min before HepaStem infusion

b) ± 2 days

c) ± 2 days with at least 7 days interval without infusion

d) ± 2 weeks

e) ± 1 month

« Before each infusion .

A : If already performed during same admission, results collected

% : On infusion day, platelets measurement to be performed prior and post infusion at 4h, 24h, 48h and 72h.

© if not already performed during same admission; if already performed, results collected

ǂ before, during, after each infusion

¥ If applicable (If already performed during same admission, results collected)

≠ : cardiac US to be performed after infusion

@ : Optionnal, only if measurement can be done locally and up to investigator's judgment

+ : On infusion day : prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion (frequency of these exams can be increased up to the investigator's judgment.)

* : Before infusion and 4h post infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

& : Prior and 24h after infusion

§ : In case of deterioration of the coagulation, these measurements will be repeated.

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome

- Liver Transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possible, probable or definite.

7.1.5. Serious Adverse Drug Reactions (SADR)

A SADR is any ADR that is serious as per the above criterias.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

<u>Possibly</u>

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probably

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definitly

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

Moderate, when interfering with ADL;

Severe, when impeding ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients

enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SADR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts each with 12 hospitalized patients in total.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

The 3 first patients infused (cohort 1a) : 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage (see section 1).

The next 3 patients' cohort will receive a lower dose of HepaStem and the next 6 patient's cohorts (high dose cohort) will receive the higher dose.

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Since it is a safety study, any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

The total sample size consideration remained unchanged with a total of 12 patients

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied.

Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], , if the patient is not able to fully understand the study based on the investigator's judgment, the informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is

initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline,

Promethera Biosciences must “verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection”.

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator’s Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient’s further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of members, all external and independent to Promethera The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.

2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.

As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort has received HepaStem infusions (complete scheme of premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patients parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a unique administration of $0.5.10^6$ cells/kg body weight).

5. The SMC will review severe coagulation events assessed as related to HepaStem administration by the investigator.
6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- One or more biostatisticians

- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists and Promethera Biosciences members may provide additional information if additional expertise is desired, but are not members of the SMC.

The frequency of SMC meetings will depend on several factors including the rate of enrollment, completion of patients in the dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL, renal replacement or use of vasopressors for Hepatorenal syndrome indication
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

Arroyo et al. 2015

11.2. CLIF ACLF GRADE

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

11.3. CLIF-C ACLF SCORE

CLIF-C ACLF = 10 × [(0,33 × CLIF OF + 0.04 × Age + 0,63 × Ln(WBC{10^9 cells/L}) – 2]

11.4. CLIF CONSORTIUM ACUTE DECOMPENSATION SCORE (CLIF-C AD)

CLIF-C AD = 10 × [(0,03 × Age {years} + 0,66 × Ln(Creatinine{mg/dL} + 1.71 × Ln(INR) + 0,88 × Ln(WBC{10^9 cells/L}) – 0,05 × Sodium {mmol/L} + 8]

Jalan et al. 2015

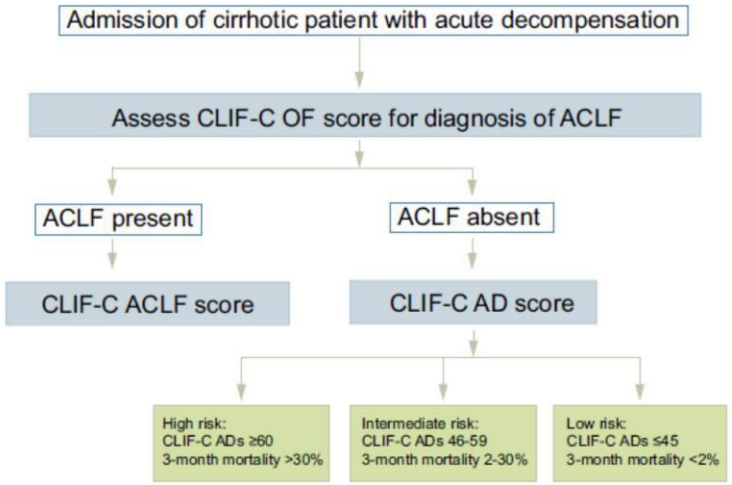

Fig. 4. Algorithm for the sequential use of the EASL-CLIF Consortium predictive scores in patients with cirrhosis admitted to hospital with acute decompensation.

11.5. MELD SCORE

MELD score is calculated using serum bilirubin, serum creatinine, and International Normalized Ratio (INR) and is given by the formula :

MELD(i) = (0.957 * ln(Serum Cr) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10 (if hemodialysis, value for Creatinine is automatically set to 4.0)

MELD Score (2016) = MELD(i) + 1.32*(137-Na) – [0.033*MELD(i)*(137-Na)]

Note: Sodium has a range of 125-137 mEq/L

The score can be calculated using online website https://www.mdcalc.com/meld-score-model-end-stage-liver-disease-12-older

11.6. CHILD PUGH SCORE

Measure	1 point	2 points	3 points
Total bilirubin, μmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8
Prothrombin time	<4.0	4.0–6.0	> 6.0

prolongation (s)			
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	Class	One year survival	Two year survival
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

Singal AK, Kamath PS. Model for End-stage Liver Disease. J Clin Exp Hepatol. 2013 Mar;3(1):50-60. Review.

11.7. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 11 May 2017

Version Number: 3.2

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____ _____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 11 May 2017

Version Number: 3.2

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competente Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

Silver Ocean Ventures SAS, CEO, represented by John Tchelingerian Promethera Biosciences	Date
Etienne Sokal, Chief Scientific & Innovation Officer Promethera Biosciences	Date
Nancy Veulemans, Vice-President Clinical & Medical Affairs Promethera Biosciences	Date
Joelle Thonnard, Head of Medical Affairs and New Indications Promethera Biosciences	Date

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 3.3 – 30 Oct 2017

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Innovation & Scientific Officer John Tchelingerian, PhD – Chief Executive Officer Nancy Veulemans, Ir – Vice President Medical and Clinical Affairs Dimitar Tonev, MD, Director Medical Affairs
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MONITORING & STUDY COORDINATION	Virginie Barthel, T.+32.471.784.097
SAFETY & PHARMACOVIGILANCE	pv@promethera.com **T.*+32.10.39.43.43***

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LIST OF ABBREVIATIONS

AD	Acute Decompensation
ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SADR	Serious Adverse Drug Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 3.3 - 30 Oct 2017
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Twelve (12) evaluable Patients
Number of Centers	5-10 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total 5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 ce
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 ce
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 ce

The next 3 patients enrolled will complete the first cohort and will receive a lower dose following SAEs observed in patient 2 and patient 3.

Six other patients will be enrolled in cohort 2.

The 3 first patients of cohort 2 (cohort 2a) will receive twice the dose compared to cohort 1b.

The 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

The statistical analysis will take into consideration the different doses applied.

Study periods

The study will recruit cirrhotic patients who are hospitalized for ACLF or Acute Decompensation at risk of developing ACLF

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Patients with Acute Decompensation of cirrhosis at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

The study is divided in the following periods: screening period, active study period divided in treatment period and evaluation period, and long-term safety follow-up.

Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

	Active study period: The active study period will last 28 days (± 2 days).The duration of the screening period plus the active period will last up to 32 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Various dose regimens of HepaStem will be given, which differ in the amount of cells per infusion and/or in the number of infusions. The 3 first patients received the dose regimen of 250.10^6 cells per infusion – this represents approximately $3.5.10^6$ cells/kg bodyweight in the first cohort. (cohort 1a) The next three patients in cohort 1 (cohort 1b) will receive a lower dose (minimum ten times lower) in a single infusion ($0.25.10^6$ cells /kg bodyweight with a maximum of 25.10^6 cells per infusion). The 3 next patients in cohort 2 (cohort 2a) will receive twice the dose of the patients in cohort 1b ($0.5.10^6$ cells/kg bodyweight with a maximum of 50.10^6 cells per infusion). The 3 next patients in cohort 2 (cohort 2b) will receive up to 2 doses of $0.5.10^6$ cells/ kg bodyweight 1 week apart ($0.5.10^6$ cells/kg bodyweight per infusion with a maximum of 50.10^6 cells per infusion). Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of members, all external and independant to Promethera Biosciences. As a minimum, the safety data will be reviewed by the SMC at the following timepoints : - For each cohort (1b, 2a and 2b), when the first evaluable patient has received the HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients. - For the first cohort (1b), when the second evaluable patient has received HepaStem infusion (complete scheme or premature stop), the patient data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients. - When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patients in the next cohort.

	In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized. If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient. More specifically, based on the patients' parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg bodyweight) instead of a single administration of $0.5.10^6$ cells/kg bodyweight). Furthermore, the 3 patients of each cohort will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete sheme or premature stop). Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period. After completion of this study, patients will be invited to be followed-up in the Patient Registry (5 years).
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and thereafter for an additional period of 1 year in the long term safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at $50x10^6$ cells/ml equivalent to $250x10^6$ cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent. In cohort 1a, 50 ml was given per infusion. For cohorts 1b, 2a and 2b, the volume of HepaStem administered will be adapted to the patient's bodyweight.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on the patient's status. The infusion rate shall not exceed 1 ml per min. For cohort 1, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion had to be respected between infusion days.

	The *Planned* schedule was : in cohort 1a, 250 million cells in 50 ml were administered on each infusion day, leading to a total of 1 billion cells ,if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone was expected to be given 15 to 30 min before each HepaStem infusion. The *Actual* schedule is: in cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2a: 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).
s	<u>Inclusion criteria</u>: 1. Adult aged between 18 and 70 year old. 2. Informed Consent. <u>N.B:</u> In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

	3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan). 4. Patient with Acute Decompensation of cirrhosis 5. Serum total Bilirubin ≥ 6 mg/dL (≥100 umol/L) 6 The INR measurement has to be : 1.2 ≤ INR < 2
Eligibility - Exclusion Criteria	<u>Exclusion criteria</u>: 1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam. 2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion. 3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion. 4. Variceal banding or sclerosis within 4 weeks before the infusion. 5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 6. Clinical evidence of Aspergillus infection. 7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion. 8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation. 9. Coagulation disorders defined as : • INR ≥ 2 • Fibrinogen < 100 mg/dL • Platelets < 50.000/mm^3 10. Major invasive procedure within 1 week before the infusion (including but not limited to transjugular liver biopsy) 11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period. 12. MELD score > 30. 13. Previous organ transplantation and/or ongoing immunosuppressive treatments. 14. Postoperative-decompensation following hepatectomy.

	15. Renal failure due to chronic kidney disease. 16. Clinically significant left-right cardiac shunt. 17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions. 18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. 19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 21. Participation to any other interventional study within the last 4 weeks. 22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisousness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28. The relationship will be assessed based on investigator assessment, and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline. <u>Secondary Endpoints</u>: • Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Mortality ○ Liver transplantation ○ Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score, Child Pugh score. • Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Bilirubin, creatinine, INR and albumin values • Long term safety follow-up ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1 ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation

	and outcome of the transplantation, New ACLF episode will be summarized at Month 3 and Year 1
Study Assessment visits	<u>Study visits</u> During the screening and treatment period, patients will be hospitalised. During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit). Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. On HepaStem infusion days,, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, TEG (if already performed as part of the clinical routine and up to investigator's judgment), coagulation factors (intrinsic and extrincsic pathway), a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day (even if HepaStem infusions are prematurily stopped). The careful evaluation of these parameters will allow or will not allow the infusion (see section 4.4 – Criteria for treatment discontinuation). On the other days during the hospital stay, patients will be followed-up according to usual practice. A study visit will be performed on Day 4, 8, 12 and 14 $\pm$ 2 days post 1st infusion, including the evaluations listed below. After the treatment period, study visits will be done on days 21 and 28 ($\pm$ 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

	After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).
	
	Up to Day 28 visit, all SAEs will be collected. After Day 28 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of the transplantation, malignancies, new AD and/or ACLF episode, AEs assessed by the investigator as possibly related to HepaStem (see Section 7.1.2).
	
	At the Month 12 study visit, patients will be invited to be included in the Patient Registry (5 years).
	
	<u>Study assessments</u>
	
	• All AEs up to Day 28
	• All AESI up to 1 Year.
	• Concomitant medication modifications up to Day 28
	• Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD score (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12,, 14, 21, 28, Months 2, 3. 6 and 12
	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
	  ○ GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
	  ○ Creatinine, Urea or BUN
	  ○ CRP
	  ○ INR, aPTT
	  ○ Serum albumin, sodium, potassium
	• Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2).

	In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment. ○ INR ○ aPTT ○ fibrinogen ○ D-Dimers ○ TEG (optional, only if measurement can be done locally and up to investigator's judgment) • Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion. • Lipase: at screening • Viral serology (HIV, HCV, HEV, HbS antigen) and Aspergilus detection: at screening (if not performed during same admission) • Urine test (Sediment, Creat, Glc, Protein, Albm): at screening • Protein C, Protein S, anti-thrombin III: at screening. In case of deterioration of the coagulation, these measurements will be repeated. • Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2b), 12, 14, 21, 28 (blood testing in central lab) • Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab) • Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab) • Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also at M12.Chest x-ray : at screening (if not performed during same admission) and at M12, • Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected) • ECG: at screening (if not performed during same admission). Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12. Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion. In case of premature withdrawal from study, an end of study visit should be perfomed if possible at the time of study withdrawal.

	In case of liver transplantation during the course of the study, a sample of the explanted liver will be collected if possible.
Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem is administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist. The 3 first patients infused (cohort 1a) received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. 2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage to a new low dose. (see section 1)). The next 3 patients will receive a lower dose of HepaStem and the next 6 patients (new high dose cohort) will receive the higher dose. The total sample size consideration remains unchanged with a total of 12 patients.
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who received at least one infusion. All study variables will be listed by treatment dose and overall.

	AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of the transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

	The Report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Period	**Screening Period**	**Active period**										
	Baseline	**Treatment Period**					**Surveillance Period**		***Long term follow-up***			
Time	**Over 1-4 days prior D1**	Infusion D1	D4[b]	D8[c]	D12[b]	D14[b]	**D21[b]**	**D28[b]**	**M2[d]**	**M3[d]**	**M6[d]**	**M12[e]**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	X	«	X	X	X	X	X	X	X	X
Vital Sign	X	↔	X	↔	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	«%	X	«%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	X	«	X	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	+	X	+	X	X	X	X	X	X	X	X
Coagulation 2 : C-Protein, S-Protein, Anti-Thrombin III	X§											
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen, D-Dimers, TEG®		+		+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	&		&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	«		«		X		X				
TEG, TG	X	*	X	*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	X	«	X	X	X	X				X
Chest X-Ray	©											X
Cardiac US Doppler	©	≠										X
ECG	©											
Blood culture or other fluid culture	A											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶ cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg)		X[a]										
Cohort 2a : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg)		X[a]										
Cohort 2b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg)		X[a]		X[a]								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a) Hydrocortisone given 15-30 min before HepaStem infusion

b) ± 2 days

c) ± 2 days with at least 7 days interval without infusion

d) ± 2 weeks

e) ± 1 month

« Before infusion .

A : If already performed during same admission, results collected

% : On infusion day, platelets measurement to be performed prior and post infusion at 4h, 24h, 48h and 72h.

© if not already performed during same admission; if already performed, results collected

↔ before, during, after infusion

¥ If applicable (If already performed during same admission, results collected)

≠ : cardiac US to be performed after infusion

@ : Optionnal, only if measurement can be done locally and up to investigator's judgment

+ : On infusion day : prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion (frequency of these exams can be increased up to the investigator's judgment.)

* : Before infusion and 4h post infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

& : Prior and 24h after infusion

§ : In case of deterioration of the coagulation, these measurements will be repeated.

1. BACKGROUND AND RATIONALE

1.1. CIRRHOSIS, ACUTE DECOMPENSATION AND ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Cirrhosis is a progressive chronic liver disease characterized by diffuse fibrosis, severe disruption of the intrahepatic venous flow, portal hypertension and liver failure. The course of cirrhosis is divided into two stages. Compensated cirrhosis defines the period between the onset of cirrhosis and the first major complication. During this period, which is relatively long in most patients (>10 years), symptoms are absent or minor, but liver lesions and portal pressure steadily progress. The term decompensated cirrhosis defines the period following the development of ascites (that is, the accumulation of large amounts of fluid within the peritoneal cavity), variceal haemorrhage and/or hepatic encephalopathy. This period is associated with short-term survival (3–5 years). It is increasingly evident that patients rarely die as a consequence of an end-stage irreversible destruction of the liver. Rather, in most patients, the cause of death is an acute deterioration in their chronic clinical condition promoted by a precipitating event — a syndrome termed acute-on-chronic liver failure (ACLF) (Arroyo et al. 2015).

It is of note that definitions on ACLF may differ worldwide. Given the heterogeneity and the importance of identifying patients four major societies/organisations have provided working definitions (APASL, NACSELD, WGO and EASL-CLIF). The common definition of ACLF is 'a syndrome characterised by acute decompensation of chronic liver disease associated with organ failure(s) and high short-term mortality'. According to the CLIF-ACLF definition developed based on the CANONIC study, ACLF is a recognised syndrome characterised by acute decompensation of cirrhosis associated with the failure of one or more organs and, in the more severe cases, system failure. The organs and systems most likely to fail are the liver, kidney, brain, coagulation, circulation and/or lungs. Patients have a high short term mortality of over 15 % at 28 days (Hernaez R et al, 2017). In the CANONIC study approximately 31% of patients admitted to a hospital for Acute Decompensation (AD) of cirrhosis had ACLF at admission (20%) or developed the syndrome during hospitalisation (11%). The common causes of acute decompensation of liver function included bacterial infections, alcoholic hepatitis, and gastrointestinal hemorrhages, but, in more than 40 % of patients, no precipitating event was identified (Moreau et al. 2013). Among patients with Acute Decompensation (AD), subgroups were identified as being at higher risk of progressing to full blown ACLF and thus at higher mortality risk (Arroyo et al. 2015).
Different grading/scoring systems have been developed in order to better determine prognosis and effectiveness of intervention and care. (Hernaez R et al, 2017).

In daily practice, MELD and Child Pugh scores are still strongly relied on to guide clinical care.

The Model for End-Stage Liver Disease, or MELD, is a scoring system for assessing the severity of chronic liver disease. This score is used by the United Network for Organ Sharing (UNOS) and Eurotransplant for prioritizing allocation of liver transplants. New MELD uses the patient's values for serum bilirubin, serum creatinine, sodium and the international normalized ratio for prothrombin time (INR) to predict survival.

Mortality and MELD score are linearly correlated amongst patients with end-stage liver disease listed for OLT with 3-month mortality estimated to be 4%, 27%, 76%, 83%, and 100% for MELD scores of <10, 10–19, 20–29, 30–39, and 40 or more respectively.

The Child–Pugh score is used in clinical practice to assess the prognosis of chronic liver disease, mainly cirrhosis. It was previously used for prioritizing allocation of liver transplants. The score employs five clinical measures of liver disease: total bilirubin, serum albumin, prothrombin time, ascites and hepatic encephalopathy. Each measure is scored 1–3, with 3 indicating most severe derangement. This leads to three Classes with one year overal survival of 100% for Class A, 81% for class B and 35% for class C. (see 11.6)

ACLF has been defined by the CLIF research consortium into four grades based on retrospectively fitting data on severity linked to mortality score (Moreau et al. 2013) (Table 1-1 and Table 1-2)

- ACLF grade 0 concerns 69.1 % of patients admitted to hospital with acute decompensation. The group is defined as no organ failure, single "non kidney" organ failure (ie, single failure of the liver, coagulation, circulation, or respiration) who had a serum creatinine level < 1.5 mg/dL and no hepatic encephalopathy, or as single cerebral failure with a serum creatinine level < 1.5 mg/dL. These patients have a 28-day and 90-day mortality rate of 4.7% and 14% respectively.
- ACLF grade 1 concerns 15.8 % of patients admitted to hospital with acute decompensation. The group is defined as single kidney failure or single non-kidney organ failure with an organ dysfunction (kidney or brain) and has a 28-day mortality rate of 23 %.
- Patients with ACLF grade 2, defined as two failing organs (10.9 % of patients admitted to hospital with acute decompensation) has an intermediate prognosis (28-day mortality rate of 31%).
- Finally, ACLF grade 3, defined as three or more organ failures (4.4 % of patients admitted to hospital with acute decompensation) has extremely high mortality rates, reaching 75 % after 28 days.

Among patients hospitalised with acute decompensation (AD) (pre ACLF according to the CLIF criteria but ACLF according to other classification systems), an analysis revealed five independent variables including age, serum sodium, white cell count, creatinine and INR as useful for defining a scoring system. The high-risk group (CLIF-C AD score > 60) and intermediate risk group (CLIF-C AD score 46-59) respectively have a 3-month mortality of over 30 % and between 2-30 %. The low risk AD group has a 3-month mortality below 2 % (Arroyo et al. 2015).

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years.The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

Cirrhotic patients with acute decompensation can only receive supportive treatments, such as antibiotics in case of infection, lactulose in case of encephalopathy, terlipressin and albumin in case of hepatorenal syndrome. However, at this moment, there are no treatments available to stop the inflammatory cascade often accompanying the acute decompensation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion,

which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 , renal replacement or use of vasopressors for Hepatorenal syndrome indication
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may cause necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver transplant rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

Conclusion on patient population: Based on this information, Promethera Biosciences proposes that the patient population is defined as cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 µmol/L) and MELD < or = 30.

Patients should have coagulation parameters within the ranges below:

- INR ≥ 1.2 and < 2
- Fibrinogen ≥ 100 mg/ dL
- Platelets ≥ 50.000/mm3

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidence supports the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properties of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells (similar cells to HHALPC) were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013).

Later, the technology of large-scale cell production was transferred to Promethera Biosciences where clinical batches of HHALPC are produced in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs).

Toxicology *in-vitro or in-vivo* studies aiming to demonstrate the safety, tolerability and tumorigenicity aspect of HepaStem were conducted. *In vivo* studies were performed in rats and mice. They included one study to assess the safety of the intravenous mode of administration. Two studies specifically assessed the risk of tumor formation as this risk has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. An *in vitro* study the pro-coagulant activity of HepaStem was confirmed (Please refer to the IB for more details).

In addition, *invitro* studies show that HepaStem cells express variable immunomodulatory surface markers of interest and have immunomodulatory functional effects: HepaStem inhibits the proliferation of activated T-lymphocytes and blocks the maturation of monocytes (see Section 1.2.6). Furthermore, 6 *in-vivo* studies were conducted with HepaStem evaluating the immunomodulatory properties using the IV route of administration and mainly doses of 12.5 10^6 cells/kg. No safety signal was detected based on these *in vivo* studies.

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg) (4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did

undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. These data laid the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

Based on literature data and Promethera experiences, it can be concluded that liver-derived MSCs, including HepaStem have a pro-coagulant activity. This pro-coagulant activity is also expressed by other MSCs. The pro-coagulant activity might be linked to tissue factor expression, an activator of the coagulation cascade. The procoagulant effect could be modulated by the concomitant administration of bivaluridin during HepaStem infusion in UCD clinical trials in order to prevent, mainly, anticipated thrombotic events. Very high cell doses have been administered intra-portally in the UCD studies in which thrombotic events only occurred at high doses (range: 115 million to 4,1 billion total cells were administered in the portal vein as a split dose in 1 to 10 infusions spread over 1 to 4 consecutive days). Bivaluridin will not be used in the ACLF clinical study as its use has not been validated for late stage cirrhotic patients. Contrary to patients with urea-cycle disorders, coagulation disturbances are common in the late stage chronic cirrhosis population and are linked to liver insufficiency. (see 1.2.5)

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

In a first-in man cohort,conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells (ADHLSC, similar cells to HHALPC) infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated this cell infusion well, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells

repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, spleen, right ankle, and spine, and had disappeared from the lungs. This is in line with the bio-distribution of another type of MSCs administrated in patients (BM-derived MSCs) that demonstrate a similar bio-distribution, with a first pass through the lung; within 24 hours, cells are mainly found in liver, spleen, kidneys and other inflamed areas, by 48 hours, more pronounced presence in the liver is observed. (NDS dossier remestemcel-L, Health Canada).

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For example, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012).

In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

It is clear from consultation of literature publications on cell therapy administration in advanced liver disease including decompensated liver cirrhosis and ACLF that doses of cell therapy protocols tended to be lower as compared to the normal range administered to patients in other immune-modulatory or anti-inflammatory protocols.

The doses and regimens administered to treat patients with chronic liver diseases, range from 0.03 over 0.5 to 1 million MSC cells/kg bodyweight. Different regimens were applied with repeated dosing up to 3 times for the lowest doses (0.03; 0,05 and 0,5 million cells/kg BW repeated 3 times). Most protocols administered the cell infusion intravenously although also other routes of administration were investigated such as intra-splenic, hepatic artery, intrahepatic, intra-lesional route of administration or central venous catheter into the femoral vein. (Berardis et al. 2015)

Based on the literature review, MSC administration is considered to be safe due to the lack of reports of significant adverse effects in the above studies, although a marked heterogeneity was observed among studies with regard to injection dose, frequency of injection, cell source, delivery route and study design. Most of these early studies reported improvements in liver function, ascites and encephalopathy.

In the first cohort of 3 patients in the HEP001 study the lowest dose (12.5×10^6 cells/kg) of the range of doses administered safely in previous studies of HepaStem in urea cycle disorder (UCD) and Crigler Najar pediatric patients was used to determine the dose in the HEP101 protocol. The (low) dose proposed (250

million cells/ infusion; ie. 3.5 10^6 cells/kg BW/infusion) was a reduction of 4x of the lowest dose tested previously (in the HEP001 protocol) and it was thought that it could be safely administered in cirrhotic patients. Additionally, the number of cells administered per infusion would be limited, similar to MSC doses given in immune mediated inflammatory diseases. In retrospect, it was clear that adaptation to the dose level similar to other MSCs given in immune-mediated inflammatory diseases was inadequate and did not take the specific case of severely ill chronic cirrhotic patients with acute decompensation into account.

Therefore, it seems that using a careful approach starting with doses commonly used in reported studies of decompensated cirrhosis and ACLF patients and published as being safe, appears to be an acceptable approach. Also, dose escalation to a maximum of 1.0 million cells/kg BW should be feasible based on a repetitive dosing schedule. In case of repetitive dosing, the doses will be given weekly, which allows time in case of fibrinolysis for the parameters to be corrected and return to normal. (please refer to rationale for changes)

1.2.6. Pre-clinical immunomodulatory data of liver-derived progenitor cells

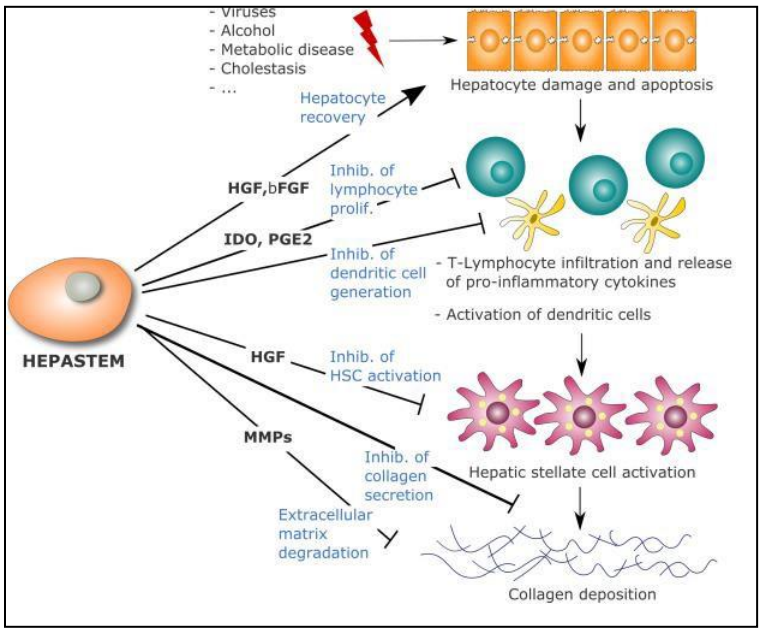
The first transcriptomics and secretomics tests performed on liver-derived progenitor cells (similar cells to HHALPC) grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (similar cells to HHALPC) (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of

HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models presents important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.7. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing to the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in

ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.8. Expected Benefits of HepaStem

Proposed mechanisms of action: after intravenous administration of HepaStem, cells are expected to circulate into the blood network where they can exert a systemic immunomodulatory action. At the same time, they have a main homing into the liver where they can thus also exert some important local immunomodulatory effects. They are expected to play their immunomodulatory roles through direct cell-to-cell interaction and through paracrine effects via the various cytokines, chemokines, MMPs and growth factors they may secrete. HepaStem could affect monocytes and DC recruitment, differentiation, maturation and function through cell contacts or paracrine signalling. HepaStem could also alter the proliferation and activation of T-lymphocytes that are another dysregulated cell type of the immune system in ACLF. In addition to modulate the behaviour of immune cells, HepaStem could modulate the proliferation and activation of hepatocytes and hepatic stellate cells and thus their secretory profiles, helping in this way the liver function recovery. The current *in vitro* and *in vivo* data, based on the scientific literature, and sponsor *in vitro* results, support all these potential immunomodulatory effects of HepaStem in ACLF patients.

Proposed clinical significant benefit: by these combined effects, HepaStem could play a favourable role in restoring an immunological balance in ACLF patients or patients at risk of ACLF, improving organ failure scores, improving clinical status, possibly leading to a resolution of this acute event and demonstrating improvement of transplantation free survival.

Considering the unmet medical need: i. the emergency to treat cirrhotic patients with Acute Decompensation (pre-ACLF or ACLF) due to the high mortality rate; ii. the shortage of healthy donors and the need of livers in the context of liver transplantation; iii. Concerns raised recently regarding artificial liver support; and iv. the mechanism of action of HepaStem, we can say that all these factors are in favour of a promising favourable benefit/risk balance for HepaStem. The exact profile of which patients will benefit most is under investigation, and also subject of this safety study.

1.2.9. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total. The study will include patients with an acute decompensation of cirrhosis and with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 30,excluding patients with circulatory,respiratory failure or severe coagulations disorders). It is planned to have a first group of 6 patients (cohort 1) being administered with the low dose.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days.

On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 patients experienced an episode of severe bleeding. Therefore, it has been decided to reduce the dose in the low dose cohort to $0.25.10^6$ cells/kg bodyweight with a maximum of 25.10^6 cells in a single infusion. A reduction of minimum 10 times the dose previously used.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

Once this has been proven safe, a second group of 3 patients (cohort 2a) will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 6 patients, appears to be an acceptable approach in patients with ACLF or with acute decompensation at risk of developing ACLF for whom no specific therapeutic or curative treatment exist. (See section 1.1)

HepaStem administration will be started rapidly after hospitalisation and will be completed within 1 day (cohort 1b) or within 1 week (cohort 2b). This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of patients with or at risk of developing ACLF. As ACLF and/or Acute Decompensation of cirrhosis is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up for safety up 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining the design of future efficacy clinical studies.

Study population

The patient population is defined by cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 30 (see section 1.1).

Patients should have coagulation parameters within the ranges below:

- INR ≥ 1.2 and < 2
- Fibrinogen ≥ 100 mg/ dL
- Platelets ≥ 50.000/mm3

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 14 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 28 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 million cells per infusion) is close to MSC doses given in other trials for immune-mediated inflammatory diseases (Section 1.2.5), therefore, it was expected to show a similar safety and efficacy profile. It corresponded also to the high dose of liver-dirived progenitor cells (similar cells to HHALPC) administered via IV to the heamophila patient (see 1.2.4).

Due to the severe bleeding that occurred in 2 of the 3 patients that received 250 10^6 cells (50 mL of HepaStem) per infusion, the next selected dose (low dose cohort 1b) will be reduced to 0.25.10^6cells/kg bodyweight (with a maximum of 25.10^6 cells per infusion) administered in a single infusion (at least a 10x reduction of the dose administered in cohort 1a).

The second selected dose represents a two-fold increase from the dose in cohort 1b (0.5.10^6cells/kg bodyweight (with a maximum of 50.10^6 cells per infusion) still in the range of doses reported for MSCs and more in the range of doses administered in the specific case of severely ill chronic liver disease patients with ACLF and acute decompensation of cirrhosis. (see1.2.5)). (for additional information, please refer to the rationale for changes).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 IU/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damage nor any respiratory symptoms, before homing mainly to the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF , it also can allow repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs also have a procoagulant activity comparable to liver-derived progenitor cells (similar cells to HHALPC) (Stephenne et al. 2012), nevertheless literature reports show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015).

However, liver failure results in a state of “rebalanced hemostasis” marked by a decrease in both pro-coagulation and anticoagulation factors. Patients with severe liver disease are not auto-anticoagulated. In essence, patients with severe liver disease, acute and/or chronic, have a tenuous rebalanced hemostasis that is easily perturbed by various disease states and concomitant medications and invasive procedures. Bleeding events including severe forms are common in these end-stage liver disease patients. The events of epistaxis and bleeding from puncture sites that occurred in 2 patients in cohort 1a (in retrospect a high dose in late stage cirrhotic patients), have been recognised in the literature as case reports. It was also stated that epistaxis as an overlooked cause of massive haematemesis in cirrhosis should be added to the list of upper GI bleeding.(Johal et al 2003). Hence, cirrhotic patients including ACLF patients are at increased risk of bleeding or thrombosis. Therefore dose reduction from normal ranges applied in other immune-modulatory diseases, modification of inclusion criteria and increased surveillance of liver and coagualation parameters is indicated.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of different regimens of HepaStem in cirrhotic patients presenting with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total.

5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next 3 patients enrolled will complete the first cohort and will receive a lower dose following SAEs observed in patient 2 and patient 3.

Six other patients will be enrolled in cohort 2.

The 3 first patients of the cohort 2 (cohort 2a) will receive twice the dose compared to the cohort 1b.

The 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

The study will recruit patients who are hospitalized for Acute Decompensation of cirrhosis and/or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

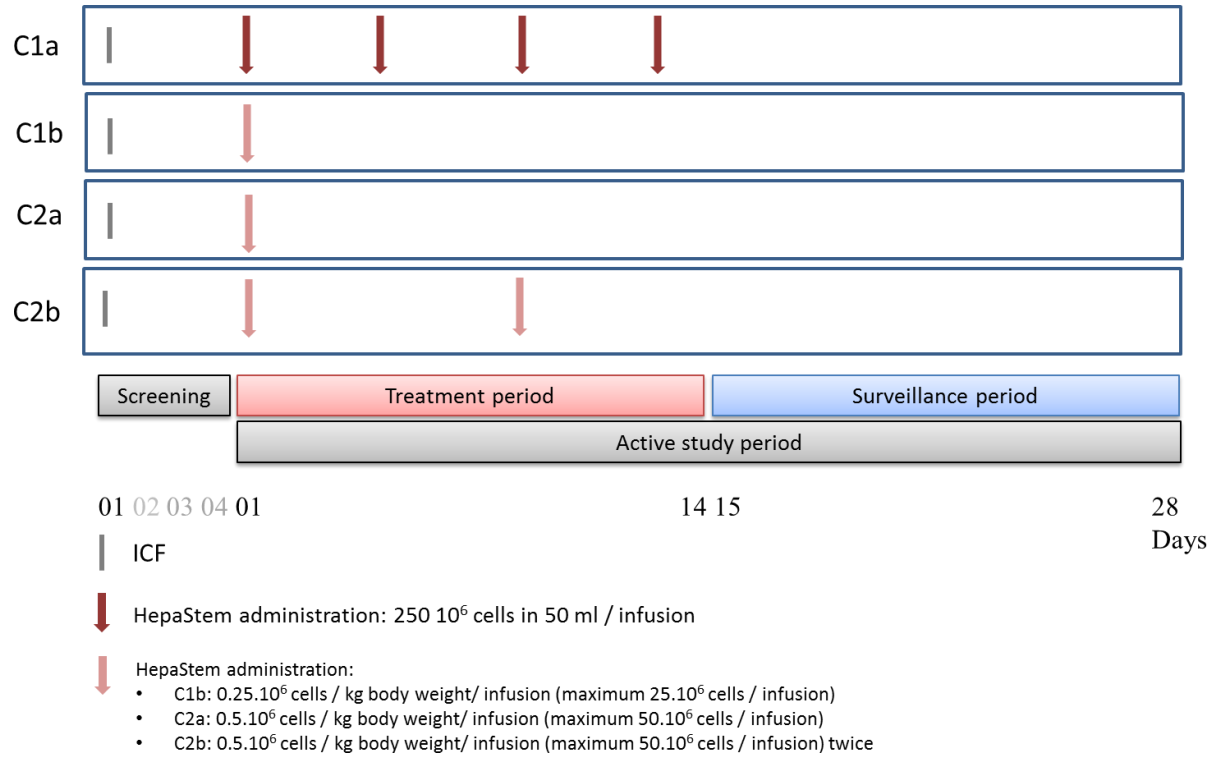
Screening period: Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days ($\pm$ 2 days). The duration of the screening period plus the active period will last up to 32 days ($\pm$ 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



Planned schedule:

For cohort 1a, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion must be respected between infusion days.

In cohort 1a, 250 million cells in 50 ml were administrated on each infusion day, leading to a total of 1 billion cells if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion.

Actual schedule:

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

Planned schedule:

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be

given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

For cohort 2a: 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation

Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of members, all external and independent to Promethera Biosciences. (See Section 9.13):

In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

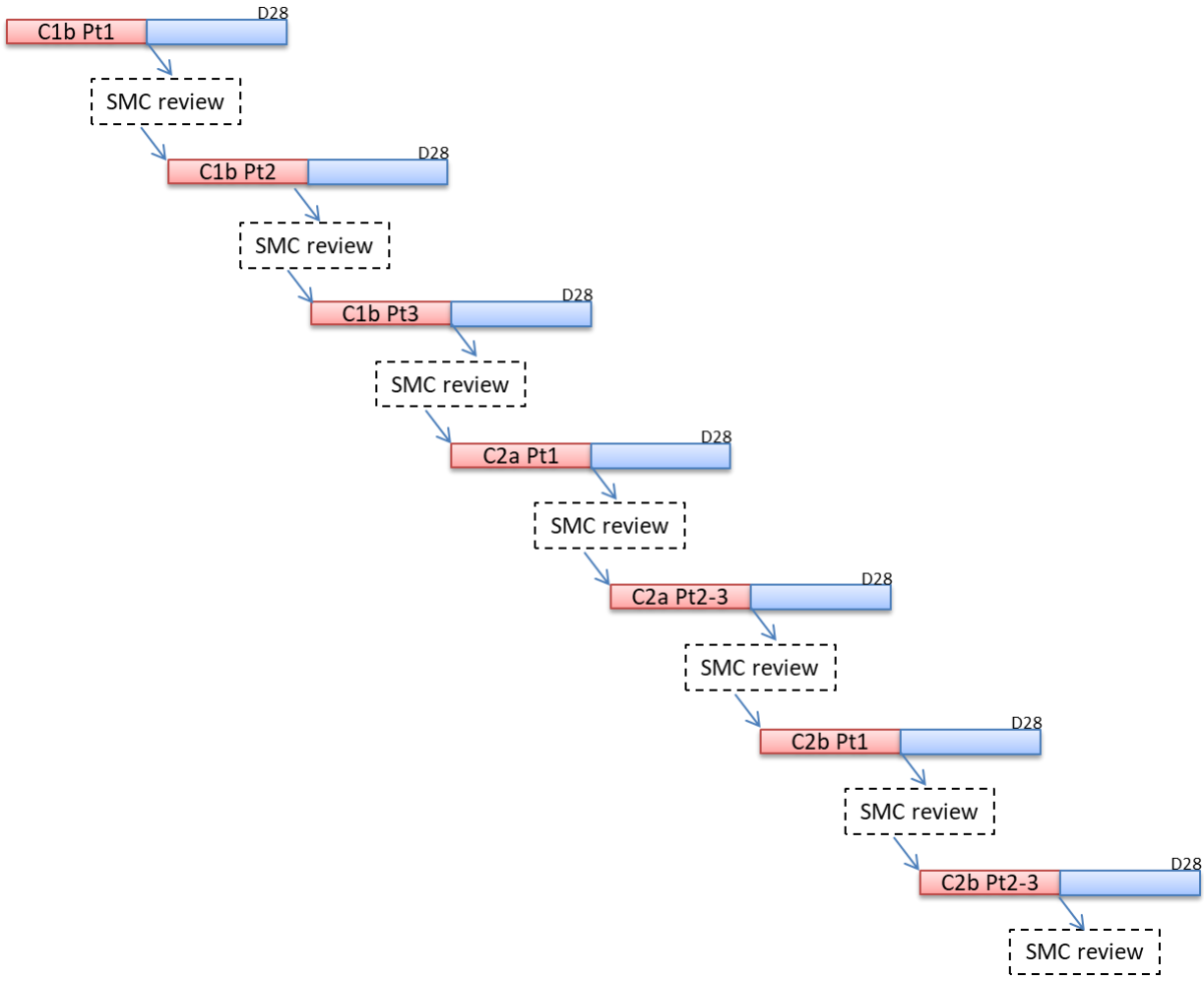
As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- For the first cohort (1b), when the second evaluable patient has received HepaStem infusion (complete scheme or premature stop), the patient data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patients' parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a single administration of $0.5.10^6$ cells/kg body weight).

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring Committe



The study assessments are described in Section 6.

Furthermore, the 3 first patients of the cohort 2a and 2b will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete scheme or premature stop). The sequential approach will be under the control of Promethera (based on review of eligibility criteria by the medical monitor and HepaStem delivery). In case of safety signal, the SMC will be involved in the AEs review and evaluation, and the SMC will advise on further inclusion.

These measures (SMC meetings and sequential treatment for the 3 first patients in each cohort) will allow respecting the progress of dose levels with limited risk for the patients.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be invited to be followed-up in the Patient Registry (5 years).

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
  - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of the transplantation
- New ACLF episode will be sumamrized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in 5 to 10 clinical centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days ($\pm$ 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. The hospitalisation unit will be adapted according to the medical status of the patient and the organization of study center hospital. Patients with a low CLIF-OF score will be more likely included in the hepatology department (standard or intermediate care unit), while the patient with high CLIF-OF score will more likely be included in the Intensive Care Unit.

Patients will remain hospitalised at least during the treatment period. During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Cirrhotic patients with Acute Decompensation at risk of developing ACLF at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last from 1 to 4 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.
2. Informed Consent.
   N.B: In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.
3. Cirrhosis as diagnosed by
   - liver histology or
   - clinical and imaging examination (may include fibroscan).
4. Patient with Acute Decompensation of cirrhosis
5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)
6. The INR measurement has to be : $1.2 \leq INR < 2$

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.

   Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.
3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion.
4. Variceal banding or sclerosis within 4 weeks before the infusion
5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
6. Clinical evidence of Aspergilus infection.
7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion.
8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
9. Coagulation disorders defined as :
   - INR ≥ 2
   - Fibrinogen < 100 mg/dL
   - Platelets < 50.000/mm^3
10. Major invasive procedure within the week before the infusion (including but not limited to tranjugular liver biopsy)
11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.
12. MELD score > 30.
13. Previous organ transplantation and/or ongoing immunosuppressive treatments.
14. Postoperative-decompensation following hepatectomy.
15. Renal failure due to chronic kidney disease.
16. Clinically significant left-right cardiac shunt.
17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.

20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
21. Participation to any other interventional study within the last 4 weeks.
22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration for a patient are the following:

- **Transitory discontinuation:** Coagulation disorders considered as significant (INR ≥ 2, Fibrinogen < 100 mg/dL, or Platelets < 50.000/mm^3) by the PI prior to each infusion should preclude the administration of Hepastem.
- Absence of portal vein flow prior to the infusion should preclude the administration of Hepastem.
- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.

Infusions may be restarted after recovery. If planned infusions are not performed within treatment period (± 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock, severe worsening of hepatic function.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAWAL CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator

- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).
- The exact dosage (volume) of Hepastem infused to the patient will be calculated based on the weight of the patient on the day of infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells/infusion (5 mL) for cohort 1b or $0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells/infusion (10 mL) for cohort 2.)
- As the exact volume to infused can be low (depending on the patient's weight), it is recommended to flush after the infusion physiological solution (NaCl 0,9%) to ensure that all the product is infused.

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and the syringe will

be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells per infusion). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapated accordingly. A single infusion is expected to last minimum 5 min (for 5 mL of reconstituted Hepastem).

For cohort 2a and 2b: 3 patients will receive HepaStem in a single infusion (cohort 2a) or in 2 repeated infusions one week apart (cohort 2b). The dosage of Hepastem per infusion will be $0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion).

Each infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single infusion is expected to last minimum 10 min (for 10 mL of reconstituted Hepastem).

The full procedure describing how to adapat the volume of HepaStem to be administrated to the patient's body weight is in the HepaStem Manual.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate,ICUs or standard unitsdepending on the medical status of the patient and the organisation of study center hospital. Regardless of the unit of hospitalization, patients will remain hospitalised at least during the treatment period, with a close monitoring of each patient. During HepaStem infusion, a continuous monitoring of the vital signs of the patient is required.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension has to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation : the syringe has to be inversed 10 times every 3 minutes.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics,risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), and risks observed with the infusion of HepaStem in the cohort 1a, main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis or to consumption of coagulation factors and subsequent bleeding; respiratory disorder as cells first transit to the lungs; hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the risks mentioned below, there might be other, at this time, unknown risks.

5.4.1. Risk and Benefit assessment

ACLF patients or patients at high risk to develop ALCF are at high mortality risk and there is currently no specific treatment for these patients. Orthotopic liver transplantation is often not a possible option for these patients. By its potential combined effects, HepaStem could play a favourable role in restoring an immunological balance in pre-ACLF / ACLF patients, leading to a resolution of this acute event and showing improvement of organ function and transplantation free survival. The main identified risks linked to HepaStem are activation of the coagulation cascade and may lead to thrombosis (observed in UCD

patients) or bleeding (observed in ACLF patients). The safety measures described below (see section 5.5) are recommended to minimize the risks of the administration of HepaStem in ACLF or pre-ACLF patients at high risk of short term mortality.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the

Before each infusion, the investigator will have to make sure the patient has the minimum criteria to receive HepaStem (see 4.4 - Criteria for study treatment discontinuation).

Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lung disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect.

Number of cells administered for the cohort 1a, 2a and 2b per infusion will be maximum 25.10^6 cells (cohort 1a) or 50.10^6 cells (cohorts 2a and 2b) and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min. The lower dose regimen will be applied before the higher one. These doses are in the very low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication and re-adjusted to the range of MSC doses given in decompensated cirrhotic and ACLF patients (See Section 1).

Furthermore, **the coagulation parameters will be closely monitored** prior and after the infusion process at 4h, 8h, 12h, 24h, 48h and 72h post infusion. (Including INR, aPTT, fibrinogen, D-Dimers, coagulation factors (pre and 24h post infusion), and TEG (optional, only if measurement can be done locally and up to investigator's judgment)

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion, and patient with risk of bleeding (defined by recent major invasive procedure, non controlled gastrointestinal hemorrhage and/or coagulation disorders) will be excluded from the study.

In case major changes in the coagulation parameters and/or clinically significant bleedings suggestive of important coagulation factors consumption occur, according to the investigator's judgement, it could be envisioned to administer coagulation factors in the form of fresh frozen plasma (FFP), coagulation factor

concentrate (ie Cofact containing Factors II, VII, IX, X plus protein S and protein C), fibrinogen concentrate (ie RiaSTAP), and/or antifibrinolytics (ie tranexamic acid). (cfr. both study patients in cohort 1a responded well to treatment with FFP and/or addition of coagulation factors).

In case of clinical signs or suspicion of pulmonary embolism, the investigator should perform exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigraphy.

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions. Any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement.

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Long Term Safety follow-up Period of 1 year..

Thereafter, patients will be invited to be followed-up Patient Registry (5 years).

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and treatment period, patients will be hospitalised in intermediate or ICUs or standard units, depending of the severity of the patient disease.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Once informed consent is signed, the screening period may last from 1 to 4 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1

On HepaStem infusion days , before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, coagulation factors, TEG (if already performed as part of the clinical routine and up to investigator's judgment) a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

The careful evaluation of these parameters will allow or not the infusion (see section 4.4 – Criteria for treatment discontinuation).

During the infusion, the patient will be continuously monitored for early detection of any potential AEs.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

A study visit will be performed on Day 14 $\pm$ 2 days, including the evaluations listed below.

In case of any suspicion of AE, the investigator will perform the exams she/he evaluates as appropriate. In particular, any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement. In case of clinical signs or suspicion of pulmonary embolism, the investigator should perfom exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigrapy.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to the 28 days visit, all SAEs will be collected. After the 28 days visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of the transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be followed-up in the Patient Registry (5 years)

6.2.1. Study assessments

- All AEs up to Day 28
- All AESI up to 1 Year.
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation),MELD score and Child Pugh will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
    - GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
    - INR
    - aPTT
    - fibrinogen

- D-Dimers
- TEG (optional, only if measurement can be done locally and up to investigator's judgment)

- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion.
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening. Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2), 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also on M12
- Chest x-ray at screening (if not performed during same admission) and at M12,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)
- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

<u>Thromboelastogram and thromboglobulin generation tests</u>

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min twice). The plasma will be colleted and put in

cryotubes (min. 2 tubes containing each min. 500 µL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Hippocrate 10,
1200 Woluwe-Saint Lambert BELGIUM

Anti-HLA antibodies

Serum will be collected and send within 48 hours (ambient temperature) to :

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Hippocrate 10 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHARTS

Table 6-1 Study Flowchart

Period	**Screening Period**	**Active period**										
	Baseline	**Treatment Period**					**Surveillance Period**		***Long term follow-up***			
Time	**Over 1-4 days prior D1**	Infusion D1	D4[b]	D8[c]	D12[b]	D14[b]	**D21[b]**	**D28[b]**	**M2[d]**	**M3[d]**	**M6[d]**	**M12[e]**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	X	«	X	X	X	X	X	X	X	X
Vital Sign	X	↔	X	↔	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	«[%]	X	«[%]	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	X	«	X	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	+	X	+	X	X	X	X	X	X	X	X
Coagulation 2 : C-Protein, S-Protein, Anti-Thrombin III	X[§]											
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen, D-Dimers, TEG@		+		+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	&		&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	«		«		X		X				
TEG, TG	X	*	X	*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	X	«	X	X	X	X				X
Chest X-Ray	©											X
Cardiac US Doppler	©	≠										X
ECG	©											
Blood culture or other fluid culture	A											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10[6] cells /kg body weight with a maximum of 25.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2a : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2b : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]		X[a]								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a) Hydrocortisone given 15-30 min before HepaStem infusion

b) ± 2 days

c) ± 2 days with at least 7 days interval without infusion

d) ± 2 weeks

e) ± 1 month

« Before infusion .

A : If already performed during same admission, results collected

% : On infusion day, platelets measurement to be performed prior and post infusion at 4h, 24h, 48h and 72h.

© if not already performed during same admission; if already performed, results collected

↔ before, during, after infusion

≠ : cardiac US to be performed after infusion

@ : Optionnal, only if measurement can be done locally and up to investigator's judgment

+ : On infusion day : prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion (frequency of these exams can be increased up to the investigator's judgment.)

* : Before infusion and 4h post infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

& : Prior and 24h after infusion

§ : In case of deterioration of the coagulation, these measurements will be repeated.

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome

- Transplantation and outcome of the transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possible, probable or definite.

7.1.5. Serious Adverse Drug Reactions (SADR)

A SADR is any ADR that is serious as per the above criterias.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

<u>Possibly</u>

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probably

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definitly

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

Moderate, when interfering with ADL;

Severe, when impeding ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients

enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SADR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts each with 12 hospitalized patients in total.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

The 3 first patients infused (cohort 1a) : 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage (see section 1).

The next 3 patients cohort will receive a lower dose of HepaStem and the next 6 patients cohorts (high dose cohort) will receive the higher dose.

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Since it is a safety study, any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

The total sample size consideration remained unchanged with a total of 12 patients

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied.

Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of thetransplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], , if the patient is not able to fully understand the study based on the investigator's judgment, the informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant

protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences

must “verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection”.

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator’s Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient’s further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of members with expertise in liver disease or other relevant medical fields, all external and independent to Promethera. The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.

2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.

As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- For the first cohort (1b), when the second evaluable patient has received HepaStem infusion (complete scheme or premature stop), the patient data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort has received HepaStem infusions (complete scheme of premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

More specifically, based on the patients parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a unique administration of $0.5.10^6$ cells/kg body weight).

5. The SMC will review severe coagulation events assessed as related to HepaStem administration by the investigator.
6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- Expert(s) in hepatology
- Expert(s) in Homeostasis
- One or more biostatisticians
- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists may be invited to participate as non-voting members at any time and Promethera Biosciences members may provide additional information if additional expertise is desired, but are not members of the SMC.

The frequency of SMC meetings will depend on several factors including the rate of enrollment, completion of patients in the dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC and to Regulatory Authority if requested.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL, renal replacement or use of vasopressors for Hepatorenal syndrome indication
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

Arroyo et al. 2015

11.2. CLIF ACLF GRADE

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

11.3. CLIF-C ACLF SCORE

CLIF-C ACLF = $10 \times [(0{,}33 \times \text{CLIF OF} + 0.04 \times \text{Age} + 0{,}63 \times \text{Ln}(\text{WBC}\{10^9 \text{ cells/L}\}) - 2]$

11.4. CLIF CONSORTIUM ACUTE DECOMPENSATION SCORE (CLIF-C AD)

CLIF-C AD = 10 × [(0,03 × Age {years} + 0,66 × Ln(Creatinine{mg/dL} + 1.71 × Ln(INR) + 0,88 × Ln(WBC{10^9 cells/L}) – 0,05 × Sodium {mmol/L} + 8]

Jalan et al. 2015

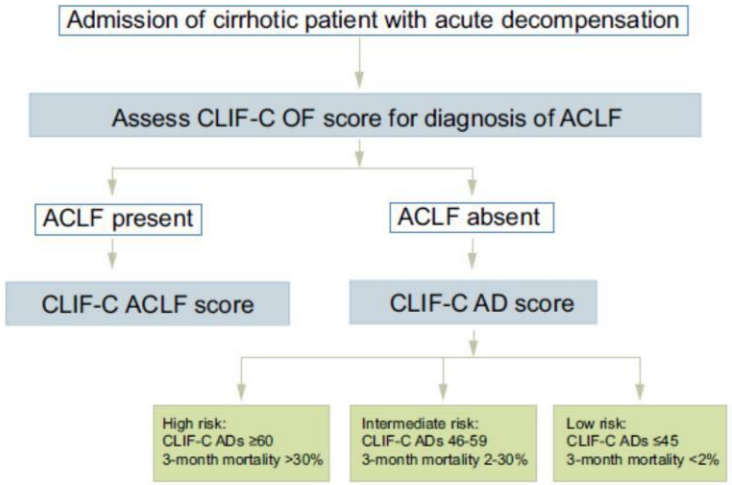

Fig. 4. Algorithm for the sequential use of the EASL-CLIF Consortium predictive scores in patients with cirrhosis admitted to hospital with acute decompensation.

11.5. MELD SCORE

MELD score is calculated using serum bilirubin, serum creatinine, and International Normalized Ratio (INR) and is given by the formula :

MELD(i) = (0.957 * ln(Serum Cr) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10 (if hemodialysis, value for Creatinine is automatically set to 4.0)

MELD Score (2016) = MELD(i) + 1.32*(137-Na) – [0.033*MELD(i)*(137-Na)]

Note: Sodium has a range of 125-137 mEq/L

11.6. THE SCORE CAN BE CALCULATED USING ONLINE WEBSITE HTTPS://WWW.MDCALC.COM/MELD-SCORE-MODEL-END-STAGE-LIVER-DISEASE-12-OLDERCHILD PUGH SCORE

Measure	1 point	2 points	3 points
Total bilirubin, μmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8
Prothrombin time prolongation (s)	<4.0	4.0–6.0	> 6.0

Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	Class	One year survival	Two year survival
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

Singal AK, Kamath PS. Model for End-stage Liver Disease. J Clin Exp Hepatol. 2013 Mar;3(1):50-60. Review.

11.7. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 30 Oct 2017

Version Number: 3.3

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____ _____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 30 Oct 2017

Version Number: 3.3

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competente Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

Silver Ocean Ventures SAS, CEO, represented by John Tchelingerian Promethera Biosciences	Date
Etienne Sokal, Chief Scientific & Innovation Officer Promethera Biosciences	Date
Nancy Veulemans, Vice-President Clinical & Medical Affairs Promethera Biosciences	Date
Dimitar Tonev, Director Medical Affairs Promethera Biosciences	Date

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 4.0 _ 15 Feb 2018

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Innovation & Scientific Officer John Tchelingerian, PhD – Chief Executive Officer Nancy Veulemans, Ir – Vice President Medical and Clinical Affairs
MONITORING & STUDY COORDINATION	Virginie Barthel, T.+32.471.784.097
SAFETY & PHARMACOVIGILANCE	pv@promethera.com **T.*+32.10.39.43.43***

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LIST OF ABBREVIATIONS

AD	Acute Decompensation
ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SADR	Serious Adverse Drug Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 4.0 – 15 Feb 2018
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Twelve (12) evaluable Patients
Number of Centers	5-10 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total 5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next 3 patients enrolled will complete the first cohort and will receive a lower dose following SAEs observed in patient 2 and patient 3.

Six other patients will be enrolled in cohort 2.

The 3 first patients of cohort 2 (cohort 2a) will receive twice the dose compared to cohort 1b.

The 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

The statistical analysis will take into consideration the different doses applied.

Study periods

The study will recruit cirrhotic patients who are hospitalized for ACLF or Acute Decompensation at risk of developing ACLF

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Patients with Acute Decompensation of cirrhosis at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining signed informed consent, the screening period may last maximum 7 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

The study is divided in the following periods: screening period, active study period divided in treatment period and evaluation period, and long-term safety follow-up.

Screening period: Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

	Active study period: The active study period will last 28 days (± 2 days).The duration of the screening period plus the active period will last up to 32 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Various dose regimens of HepaStem will be given, which differ in the amount of cells per infusion and/or in the number of infusions. The 3 first patients received the dose regimen of 250.10^6 cells per infusion – this represents approximately $3.5.10^6$ cells/kg bodyweight in the first cohort. (cohort 1a) The next three patients in cohort 1 (cohort 1b) will receive a lower dose (minimum ten times lower) in a single infusion ($0.25.10^6$ cells /kg bodyweight with a maximum of 25.10^6 cells per infusion). The 3 next patients in cohort 2 (cohort 2a) will receive twice the dose of the patients in cohort 1b ($0.5.10^6$ cells/kg bodyweight with a maximum of 50.10^6 cells per infusion). The 3 next patients in cohort 2 (cohort 2b) will receive up to 2 doses of $0.5.10^6$ cells/ kg bodyweight 1 week apart ($0.5.10^6$ cells/kg bodyweight per infusion with a maximum of 50.10^6 cells per infusion). Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of members, all external and independent to Promethera Biosciences. As a minimum, the safety data will be reviewed by the SMC at the following timepoints : - For each cohort (1b, 2a and 2b), when the first evaluable patient has received the HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients. - When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advice on the enrolment of patients in the next cohort.

	If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient. More specifically, based on the patients' parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg bodyweight) instead of a single administration of $0.5.10^6$ cells/kg bodyweight). Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period. After completion of this study, patients will be followed-up in the Patient Registry.
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days (± 2 days) in the screening plus active treatment period), and thereafter for an additional period of 1 year in the long term safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at $50x10^6$ cells/ml equivalent to $250x10^6$ cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent. In cohort 1a, 50 ml was given per infusion. For cohorts 1b, 2a and 2b, the volume of HepaStem administered will be adapted to the patient's bodyweight.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on the patient's status. The infusion rate shall not exceed 1 ml per min. For cohort 1, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion had to be respected between infusion days. The *Planned* schedule was : in cohort 1a, 250 million cells in 50 ml were administered on each infusion day, leading to a total of 1 billion cells ,if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone was expected to be given 15 to 30 min before each HepaStem infusion.

	The *Actual* schedule is: in cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2a: 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).
Eligibility - Inclusion Criteria	<u>Inclusion criteria</u>: 1. Adult aged between 18 and 70 year old. 2. Signed Informed Consent. <u>N.B:</u> In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement. 3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan). 4. Patient with Acute Decompensation of cirrhosis

	5. Serum total Bilirubin ≥ 6 mg/dL (≥100 umol/L) 6 The INR measurement has to be : 1.2 ≤ INR < 2
Eligibility - Exclusion Criteria	<u>Exclusion criteria</u>: 1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam. 2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion. 3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion. 4. Variceal banding or sclerosis within 4 weeks before the infusion. 5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 6. Clinical evidence of Aspergillus infection. 7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion. 8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation. 9. Coagulation disorders defined as : • INR ≥ 2 • Fibrinogen < 100 mg/dL • Platelets < 50.000/mm^3 10. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator. 11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period. 12. MELD score > 30. 13. Previous organ transplantation and/or ongoing immunosuppressive treatments. 14. Postoperative-decompensation following hepatectomy.

	15. Renal failure due to chronic kidney disease. 16. Clinically significant left-right cardiac shunt. 17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions. 18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. 19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 21. Participation to any other interventional study within the last 4 weeks. 22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisousness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28. The relationship will be assessed based on investigator assessment, and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline. <u>Secondary Endpoints</u>: • Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Mortality ○ Liver transplantation ○ Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score, Child Pugh score. • Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Bilirubin, creatinine, INR and albumin values • Long term safety follow-up

	○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1 ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation New ACLF episode will be summarized at Month 3 and Year 1
Study Assessment visits	<u>Study visits</u> During the screening and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. On HepaStem infusion days,, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, TEG (if already performed as part of the clinical routine and up to investigator's judgment), coagulation factors (intrinsic and extrincsic pathway), a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day (even if HepaStem infusions are prematurily stopped). The careful evaluation of these parameters will allow or will not allow the infusion (see section 4.4 – Criteria for treatment discontinuation). On the other days during the hospital stay, patients will be followed-up according to usual practice. A study visit will be performed on Day 4, 8, 12 and 14 $\pm$ 2 days post 1st infusion, including the evaluations listed below. After the treatment period, study visits will be done on days 21 and 28 ($\pm$2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

	After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).
	
	Up to Day 28 visit, all SAEs will be collected. After Day 28 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new AD and/or ACLF episode, AEs assessed by the investigator as possibly related to HepaStem (see Section 7.1.2).
	
	At Month 12 study visit, patients will be invited to be included in the Patient Registry.
	
	<u>Study assessments</u>
	
	• All AEs up to Day 28
	• All AESI up to 1 Year.
	• Concomitant medication modifications up to Day 28
	• Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD score (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12,, 14, 21, 28, Months 2, 3. 6 and 12
	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
	  ○ GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
	  ○ Creatinine, Urea or BUN
	  ○ CRP
	  ○ INR, aPTT
	  ○ Serum albumin, sodium, potassium
	• Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day

	8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment. ○ INR ○ aPTT ○ fibrinogen ○ D-Dimers ○ TEG (optional, only if measurement can be done locally and up to investigator's judgment) • Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion. • Lipase: at screening • Viral serology (HIV, HCV, HEV, HbS antigen) and Aspergilus detection: at screening (if not performed during same admission) • Urine test (Sediment, Creat, Glc, Protein, Albm): at screening • Protein C, Protein S, anti-thrombin III: at screening. In case of deterioration of the coagulation, these measurements will be repeated. • Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2b), 12, 14, 21, 28 (blood testing in central lab) • Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab) • Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab) • Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also at M12. • Chest x-ray : at screening (if not performed during same admission) and at M12, • Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected) • ECG: at screening (if not performed during same admission). • Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12. A SMC will review safety data and advise on study conduct.

	Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion. In case of premature withdrawal from study, an end of study visit should be perfomed if possible at the time of study withdrawal. In case of liver transplantation during the course of the study, a sample of the explanted liver will be collected if possible.
Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem is administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist. The 3 first patients infused (cohort 1a) received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. 2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage to a new low dose. (see section 1)). The next 3 patients will receive a lower dose of HepaStem and the next 6 patients (new high dose cohort) will receive the higher dose. The total sample size consideration remains unchanged with a total of 12 patients.

Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who received at least one infusion. All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

	The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The Report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Period	**Screening Period**	**Active period**							***Long term follow-up***			
	Baseline	**Treatment Period**					**Surveillance Period**					
Time	**Maximum 7 days prior D1**	Infusion D1	D4[b]	D8[c]	D12[b]	D14[b]	**D21[b]**	**D28[b]**	**M2[d]**	**M3[d]**	**M6[d]**	**M12[e]**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	X	«	X	X	X	X	X	X	X	X
Vital Sign	X	↔	X	↔	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	«[%]	X	«[%]	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	X	«	X	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	+	X	+	X	X	X	X	X	X	X	X
Coagulation 2 : C-Protein, S-Protein, Anti-Thrombin III	X[§]											
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen, D-Dimers, TEG@		+		+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	&		&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	«		«		X		X				
TEG, TG	X	*	X	*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	X	«	X	X	X	X				X
Chest X-Ray	©											X
Cardiac US Doppler	©	≠										X
ECG	©											
Blood culture or other fluid culture	A											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶ cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg)		X[a]										
Cohort 2a : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg)		X[a]										
Cohort 2b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg)		X[a]		X[a]								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a) Hydrocortisone given 15-30 min before HepaStem infusion

b) ± 2 days

c) ± 2 days with at least 7 days interval without infusion

d) ± 2 weeks

e) ± 1 month

« Before infusion .

A : If already performed during same admission, results collected

% : On infusion day, platelets measurement to be performed prior and post infusion at 1h, 24h, 48h and 72h

© if not already performed during same admission; if already performed, results collected

↔ before, during, after infusion

≠ : cardiac US to be performed after infusion

@ : Optionnal, only if measurement can be done locally and up to investigator's judgment

+ : On infusion day : prior and 1h, 3h, 5h, 8h, 12h, 18h, 24h, 48h and 72h post infusion (frequency of these exams can be increased up to the investigator's judgment.)

* : Before infusion and 3h post infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

& : Prior and 24h after infusion

§ : In case of deterioration of the coagulation, these measurements will be repeated.

1. BACKGROUND AND RATIONALE

1.1. CIRRHOSIS, ACUTE DECOMPENSATION AND ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Cirrhosis is a progressive chronic liver disease characterized by diffuse fibrosis, severe disruption of the intrahepatic venous flow, portal hypertension and liver failure. The course of cirrhosis is divided into two stages. Compensated cirrhosis defines the period between the onset of cirrhosis and the first major complication. During this period, which is relatively long in most patients (>10 years), symptoms are absent or minor, but liver lesions and portal pressure steadily progress. The term decompensated cirrhosis defines the period following the development of ascites (that is, the accumulation of large amounts of fluid within the peritoneal cavity), variceal haemorrhage and/or hepatic encephalopathy. This period is associated with short-term survival (3–5 years). It is increasingly evident that patients rarely die as a consequence of an end-stage irreversible destruction of the liver. Rather, in most patients, the cause of death is an acute deterioration in their chronic clinical condition promoted by a precipitating event — a syndrome termed acute-on-chronic liver failure (ACLF) (Arroyo et al. 2015).

It is of note that definitions on ACLF may differ worldwide. Given the heterogeneity and the importance of identifying patients four major societies/organisations have provided working definitions (APASL, NACSELD, WGO and EASL-CLIF). The common definition of ACLF is 'a syndrome characterised by acute decompensation of chronic liver disease associated with organ failure(s) and high short-term mortality'. According to the CLIF-ACLF definition developed based on the CANONIC study, ACLF is a recognised syndrome characterised by acute decompensation of cirrhosis associated with the failure of one or more organs and, in the more severe cases, system failure. The organs and systems most likely to fail are the liver, kidney, brain, coagulation, circulation and/or lungs. Patients have a high short term mortality of over 15 % at 28 days (Hernaez R et al, 2017). In the CANONIC study approximately 31% of patients admitted to a hospital for Acute Decompensation (AD) of cirrhosis had ACLF at admission (20%) or developed the syndrome during hospitalisation (11%). The common causes of acute decompensation of liver function included bacterial infections, alcoholic hepatitis, and gastrointestinal hemorrhages, but, in more than 40 % of patients, no precipitating event was identified (Moreau et al. 2013). Among patients with Acute Decompensation (AD), subgroups were identified as being at higher risk of progressing to full blown ACLF and thus at higher mortality risk (Arroyo et al. 2015).
Different grading/scoring systems have been developed in order to better determine prognosis and effectiveness of intervention and care. (Hernaez R et al, 2017).

In daily practice, MELD and Child Pugh scores are still strongly relied on to guide clinical care.

The Model for End-Stage Liver Disease, or MELD, is a scoring system for assessing the severity of chronic liver disease. This score is used by the United Network for Organ Sharing (UNOS) and Eurotransplant for prioritizing allocation of liver transplants. New MELD uses the patient's values for serum bilirubin, serum creatinine, sodium and the international normalized ratio for prothrombin time (INR) to predict survival.

Mortality and MELD score are linearly correlated amongst patients with end-stage liver disease listed for OLT with 3-month mortality estimated to be 4%, 27%, 76%, 83%, and 100% for MELD scores of <10, 10–19, 20–29, 30–39, and 40 or more respectively.

The Child–Pugh score is used in clinical practice to assess the prognosis of chronic liver disease, mainly cirrhosis. It was previously used for prioritizing allocation of liver transplants. The score employs five clinical measures of liver disease: total bilirubin, serum albumin, prothrombin time, ascites and hepatic encephalopathy. Each measure is scored 1–3, with 3 indicating most severe derangement. This leads to three Classes with one year overal survival of 100% for Class A, 81% for class B and 35% for class C. (see 0)

ACLF has been defined by the CLIF research consortium into four grades based on retrospectively fitting data on severity linked to mortality score (Moreau et al. 2013) (Table 1-1 and Table 1-2)

- ACLF grade 0 concerns 69.1 % of patients admitted to hospital with acute decompensation. The group is defined as no organ failure, single "non kidney" organ failure (ie, single failure of the liver, coagulation, circulation, or respiration) who had a serum creatinine level < 1.5 mg/dL and no hepatic encephalopathy, or as single cerebral failure with a serum creatinine level < 1.5 mg/dL. These patients have a 28-day and 90-day mortality rate of 4.7% and 14% respectively.
- ACLF grade 1 concerns 15.8 % of patients admitted to hospital with acute decompensation. The group is defined as single kidney failure or single non-kidney organ failure with an organ dysfunction (kidney or brain) and has a 28-day mortality rate of 23 %.
- Patients with ACLF grade 2, defined as two failing organs (10.9 % of patients admitted to hospital with acute decompensation) has an intermediate prognosis (28-day mortality rate of 31%).
- Finally, ACLF grade 3, defined as three or more organ failures (4.4 % of patients admitted to hospital with acute decompensation) has extremely high mortality rates, reaching 75 % after 28 days.

Among patients hospitalised with acute decompensation (AD) (pre ACLF according to the CLIF criteria but ACLF according to other classification systems), an analysis revealed five independent variables including age, serum sodium, white cell count, creatinine and INR as useful for defining a scoring system. The high-risk group (CLIF-C AD score > 60) and intermediate risk group (CLIF-C AD score 46-59) respectively have a 3-month mortality of over 30 % and between 2-30 %. The low risk AD group has a 3-month mortality below 2 % (Arroyo et al. 2015).

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years.The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

Cirrhotic patients with acute decompensation can only receive supportive treatments, such as antibiotics in case of infection, lactulose in case of encephalopathy, terlipressin and albumin in case of hepatorenal syndrome. However, at this moment, there are no treatments available to stop the inflammatory cascade often accompanying the acute decompensation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion,

which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 , renal replacement or use of vasopressors for Hepatorenal syndrome indication
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may cause necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver transplant rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

Conclusion on patient population: Based on this information, Promethera Biosciences proposes that the patient population is defined as cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 µmol/L) and MELD < or = 30.

Patients should have coagulation parameters within the ranges below:

- INR ≥ 1.2 and < 2
- Fibrinogen ≥ 100 mg/ dL
- Platelets ≥ 50.000/mm3

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidence supports the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properties of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells (similar cells to HHALPC) were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013).

Later, the technology of large-scale cell production was transferred to Promethera Biosciences where clinical batches of HHALPC are produced in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs).

Toxicology *in-vitro or in-vivo* studies aiming to demonstrate the safety, tolerability and tumorigenicity aspect of HepaStem were conducted. *In vivo* studies were performed in rats and mice. They included one study to assess the safety of the intravenous mode of administration. Two studies specifically assessed the risk of tumor formation as this risk has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. An *in vitro* study the pro-coagulant activity of HepaStem was confirmed (Please refer to the IB for more details).

In addition, *invitro* studies show that HepaStem cells express variable immunomodulatory surface markers of interest and have immunomodulatory functional effects: HepaStem inhibits the proliferation of activated T-lymphocytes and blocks the maturation of monocytes (see Section 1.2.6). Furthermore, 6 *in-vivo* studies were conducted with HepaStem evaluating the immunomodulatory properties using the IV route of administration and mainly doses of 12.5 10^6 cells/kg. No safety signal was detected based on these *in vivo* studies.

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg) (4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did

undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. These data laid the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

Based on literature data and Promethera experiences, it can be concluded that liver-derived MSCs, including HepaStem have a pro-coagulant activity. This pro-coagulant activity is also expressed by other MSCs. The pro-coagulant activity might be linked to tissue factor expression, an activator of the coagulation cascade. The procoagulant effect could be modulated by the concomitant administration of bivaluridin during HepaStem infusion in UCD clinical trials in order to prevent, mainly, anticipated thrombotic events. Very high cell doses have been administered intra-portally in the UCD studies in which thrombotic events only occurred at high doses (range: 115 million to 4,1 billion total cells were administered in the portal vein as a split dose in 1 to 10 infusions spread over 1 to 4 consecutive days). Bivaluridin will not be used in the ACLF clinical study as its use has not been validated for late stage cirrhotic patients. Contrary to patients with urea-cycle disorders, coagulation disturbances are common in the late stage chronic cirrhosis population and are linked to liver insufficiency. (see 1.2.5)

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

In a first-in man cohort conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells (ADHLSC, similar cells to HHALPC) infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated this cell infusion well, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells

repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, spleen, right ankle, and spine, and had disappeared from the lungs. This is in line with the bio-distribution of another type of MSCs administrated in patients (BM-derived MSCs) that demonstrate a similar bio-distribution, with a first pass through the lung; within 24 hours, cells are mainly found in liver, spleen, kidneys and other inflamed areas, by 48 hours, more pronounced presence in the liver is observed. (NDS dossier remestemcel-L, Health Canada).

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For example, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012).

In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

It is clear from consultation of literature publications on cell therapy administration in advanced liver disease including decompensated liver cirrhosis and ACLF that doses of cell therapy protocols tended to be lower as compared to the normal range administered to patients in other immune-modulatory or anti-inflammatory protocols.

The doses and regimens administered to treat patients with chronic liver diseases, range from 0.03 over 0.5 to 1 million MSC cells/kg bodyweight. Different regimens were applied with repeated dosing up to 3 times for the lowest doses (0.03; 0,05 and 0,5 million cells/kg BW repeated 3 times). Most protocols administered the cell infusion intravenously although also other routes of administration were investigated such as intra-splenic, hepatic artery, intrahepatic, intra-lesional route of administration or central venous catheter into the femoral vein. (Berardis et al. 2015)

Based on the literature review, MSC administration is considered to be safe due to the lack of reports of significant adverse effects in the above studies, although a marked heterogeneity was observed among studies with regard to injection dose, frequency of injection, cell source, delivery route and study design. Most of these early studies reported improvements in liver function, ascites and encephalopathy.

In the first cohort of 3 patients in the HEP001 study the lowest dose (12.5×10^6 cells/kg) of the range of doses administered safely in previous studies of HepaStem in urea cycle disorder (UCD) and Crigler Najar pediatric patients was used to determine the dose in the HEP101 protocol. The (low) dose proposed (250

million cells/ infusion; ie. 3.5 10^6 cells/kg BW/infusion) was a reduction of 4x of the lowest dose tested previously (in the HEP001 protocol) and it was thought that it could be safely administered in cirrhotic patients. Additionally, the number of cells administered per infusion would be limited, similar to MSC doses given in immune mediated inflammatory diseases. In retrospect, it was clear that adaptation to the dose level similar to other MSCs given in immune-mediated inflammatory diseases was inadequate and did not take the specific case of severely ill chronic cirrhotic patients with acute decompensation into account.

Therefore, it seems that using a careful approach starting with doses commonly used in reported studies of decompensated cirrhosis and ACLF patients and published as being safe, appears to be an acceptable approach. Also, dose escalation to a maximum of 1.0 million cells/kg BW should be feasible based on a repetitive dosing schedule. In case of repetitive dosing, the doses will be given weekly, which allows time in case of fibrinolysis for the parameters to be corrected and return to normal. (please refer to rationale for changes)

1.2.6. Pre-clinical immunomodulatory data of liver-derived progenitor cells

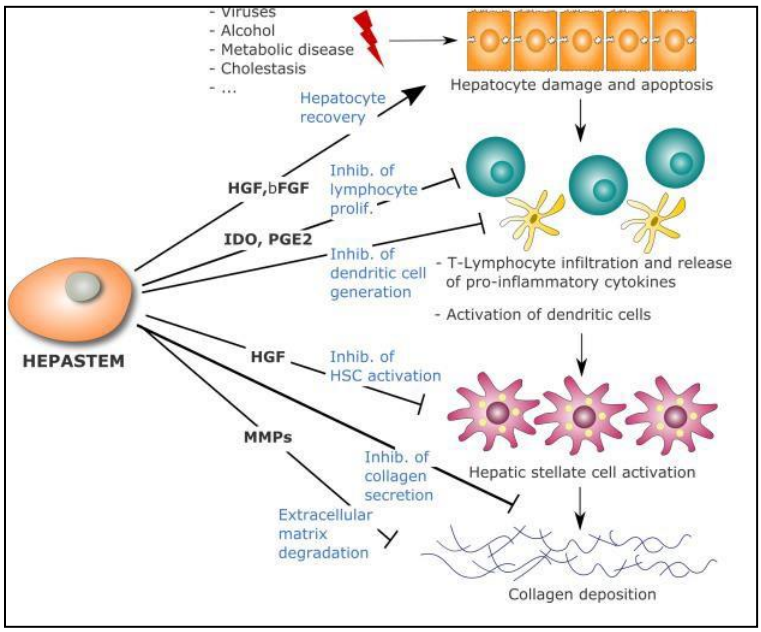
The first transcriptomics and secretomics tests performed on liver-derived progenitor cells (similar cells to HHALPC) grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (similar cells to HHALPC) (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of

HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models presents important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.7. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing to the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in

ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.8. Expected Benefits of HepaStem

Proposed mechanisms of action: after intravenous administration of HepaStem, cells are expected to circulate into the blood network where they can exert a systemic immunomodulatory action. At the same time, they have a main homing into the liver where they can thus also exert some important local immunomodulatory effects. They are expected to play their immunomodulatory roles through direct cell-to-cell interaction and through paracrine effects via the various cytokines, chemokines, MMPs and growth factors they may secrete. HepaStem could affect monocytes and DC recruitment, differentiation, maturation and function through cell contacts or paracrine signalling. HepaStem could also alter the proliferation and activation of T-lymphocytes that are another dysregulated cell type of the immune system in ACLF. In addition to modulate the behaviour of immune cells, HepaStem could modulate the proliferation and activation of hepatocytes and hepatic stellate cells and thus their secretory profiles, helping in this way the liver function recovery. The current *in vitro* and *in vivo* data, based on the scientific literature, and sponsor *in vitro* results, support all these potential immunomodulatory effects of HepaStem in ACLF patients.

Proposed clinical significant benefit: by these combined effects, HepaStem could play a favourable role in restoring an immunological balance in ACLF patients or patients at risk of ACLF, improving organ failure scores, improving clinical status, possibly leading to a resolution of this acute event and demonstrating improvement of transplantation free survival.

Considering the unmet medical need: i. the emergency to treat cirrhotic patients with Acute Decompensation (pre-ACLF or ACLF) due to the high mortality rate; ii. the shortage of healthy donors and the need of livers in the context of liver transplantation; iii. Concerns raised recently regarding artificial liver support; and iv. the mechanism of action of HepaStem, we can say that all these factors are in favour of a promising favourable benefit/risk balance for HepaStem. The exact profile of which patients will benefit most is under investigation, and also subject of this safety study.

1.2.9. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total. The study will include patients with an acute decompensation of cirrhosis and with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 30,excluding patients with circulatory,respiratory failure or severe coagulations disorders). It is planned to have a first group of 6 patients (cohort 1) being administered with the low dose.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days.

On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 patients experienced an episode of severe bleeding. Therefore, it has been decided to reduce the dose in the low dose cohort to $0.25.10^6$ cells/kg bodyweight with a maximum of 25.10^6 cells in a single infusion. A reduction of minimum 10 times the dose previously used.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

Once this has been proven safe, a second group of 3 patients (cohort 2a) will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 6 patients, appears to be an acceptable approach in patients with ACLF or with acute decompensation at risk of developing ACLF for whom no specific therapeutic or curative treatment exist. (See section 1.1)

HepaStem administration will be started rapidly after hospitalisation and will be completed within 1 day (cohort 1b) or within 1 week (cohort 2b). This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of patients with or at risk of developing ACLF. As ACLF and/or Acute Decompensation of cirrhosis is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up for safety up 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining the design of future efficacy clinical studies.

Study population

The patient population is defined by cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 30 (see section 1.1).

Patients should have coagulation parameters within the ranges below:

- INR ≥ 1.2 and < 2
- Fibrinogen ≥ 100 mg/ dL
- Platelets ≥ 50.000/mm3

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 14 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 28 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 million cells per infusion) is close to MSC doses given in other trials for immune-mediated inflammatory diseases (Section 1.2.5), therefore, it was expected to show a similar safety and efficacy profile. It corresponded also to the high dose of liver-dirived progenitor cells (similar cells to HHALPC) administered via IV to the heamophila patient (see 1.2.4).

Due to the severe bleeding that occurred in 2 of the 3 patients that received 250 10^6 cells (50 mL of HepaStem) per infusion, the next selected dose (low dose cohort 1b) will be reduced to 0.25.10^6cells/kg bodyweight (with a maximum of 25.10^6 cells per infusion) administered in a single infusion (at least a 10x reduction of the dose administered in cohort 1a).

The second selected dose represents a two-fold increase from the dose in cohort 1b (0.5.10^6cells/kg bodyweight (with a maximum of 50.10^6 cells per infusion) still in the range of doses reported for MSCs and more in the range of doses administered in the specific case of severely ill chronic liver disease patients with ACLF and acute decompensation of cirrhosis. (see1.2.5)). (for additional information, please refer to the rationale for changes).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 IU/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damage nor any respiratory symptoms, before homing mainly to the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF , it also can allow repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs also have a procoagulant activity comparable to liver-derived progenitor cells (similar cells to HHALPC) (Stephenne et al. 2012), nevertheless literature reports show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015).

However, liver failure results in a state of “rebalanced hemostasis” marked by a decrease in both pro-coagulation and anticoagulation factors. Patients with severe liver disease are not auto-anticoagulated. In essence, patients with severe liver disease, acute and/or chronic, have a tenuous rebalanced hemostasis that is easily perturbed by various disease states and concomitant medications and invasive procedures. Bleeding events including severe forms are common in these end-stage liver disease patients. The events of epistaxis and bleeding from puncture sites that occurred in 2 patients in cohort 1a (in retrospect a high dose in late stage cirrhotic patients), have been recognised in the literature as case reports. It was also stated that epistaxis as an overlooked cause of massive haematemesis in cirrhosis should be added to the list of upper GI bleeding.(Johal et al 2003). Hence, cirrhotic patients including ACLF patients are at increased risk of bleeding or thrombosis. Therefore dose reduction from normal ranges applied in other immune-modulatory diseases, modification of inclusion criteria and increased surveillance of liver and coagualation parameters is indicated.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of different regimens of HepaStem in cirrhotic patients presenting with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total.

5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next 3 patients enrolled will complete the first cohort and will receive a lower dose following SAEs observed in patient 2 and patient 3.

Six other patients will be enrolled in cohort 2.

The 3 first patients of the cohort 2 (cohort 2a) will receive twice the dose compared to the cohort 1b.

The 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

The study will recruit patients who are hospitalized for Acute Decompensation of cirrhosis and/or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

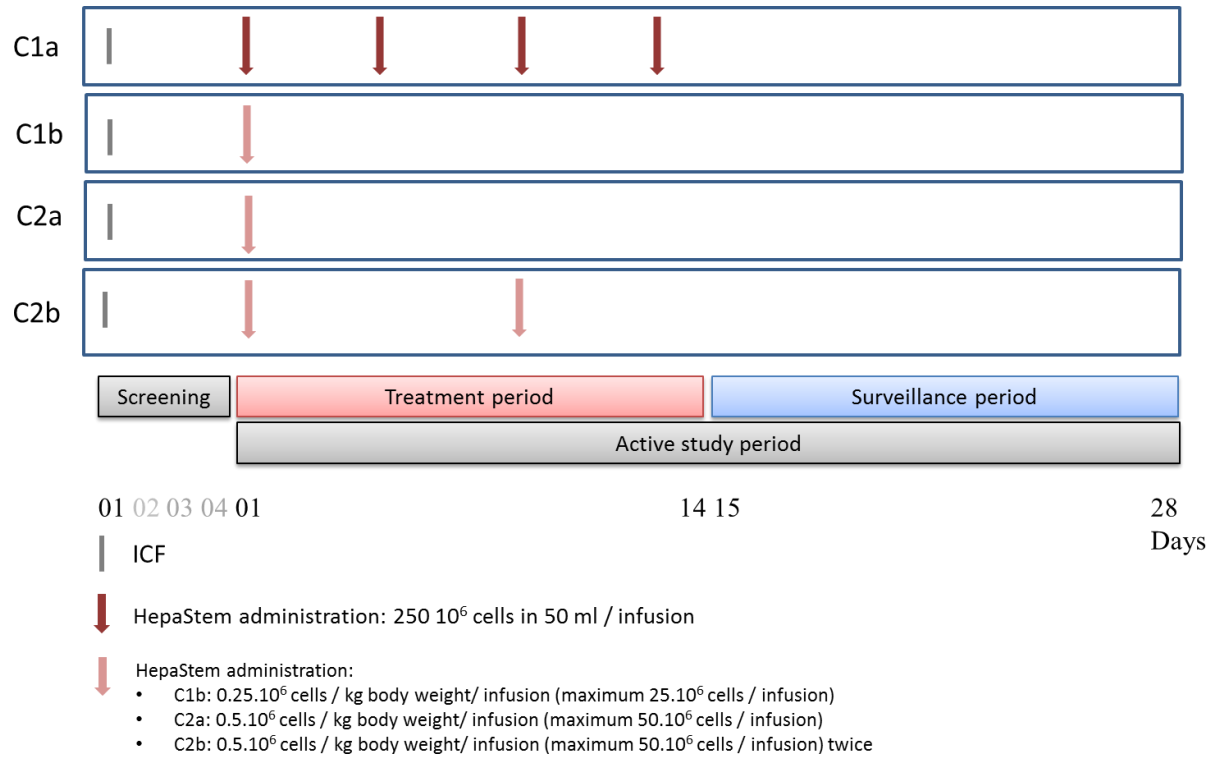
Screening period: Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days ($\pm$ 2 days). The duration of the screening period plus the active period will last up to 32 days ($\pm$ 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



Planned schedule:

For cohort 1a, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion must be respected between infusion days.

In cohort 1a, 250 million cells in 50 ml were administrated on each infusion day, leading to a total of 1 billion cells if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion.

Actual schedule:

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

Planned schedule:

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

For cohort 2a: 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of external members and Promethera members (See Section 9.13):

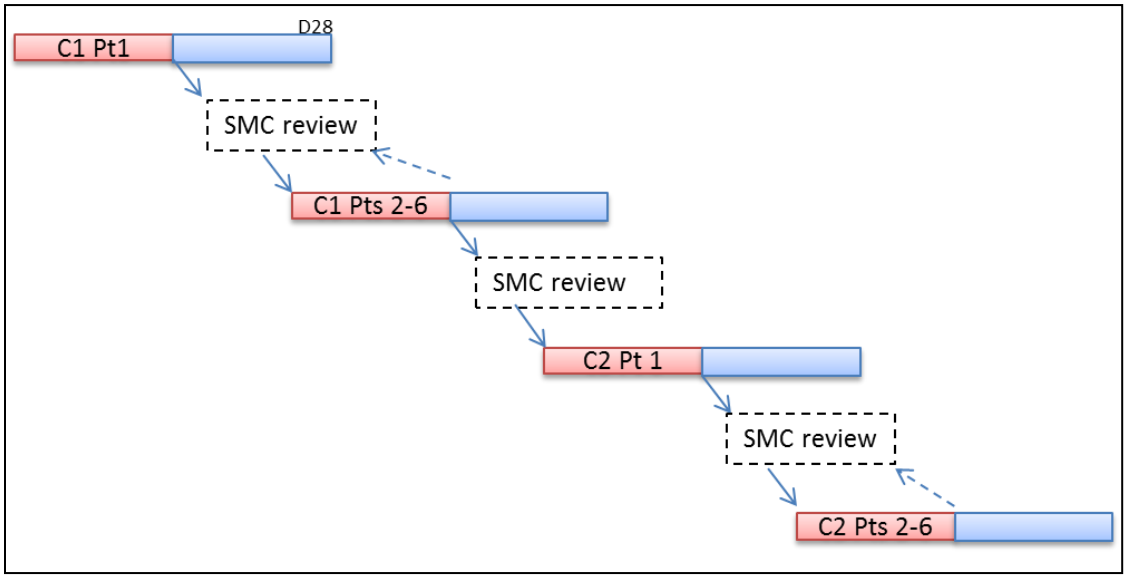
As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patients parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a single administration of $0.5.10^6$ cells/kg body weight).

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring Committe



The study assessments are described in Section 6.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be followed-up in the Patient Registry.

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
    - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation
- New ACLF episode will be sumamrized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in 5 to 10 clinical centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (32 days ($\pm$ 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the long term safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Cirrhotic patients with Acute Decompensation at risk of developing ACLF at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining signed informed consent, the screening period may last maximum 7 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.
2. Signed Informed Consent.
   N.B: In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.
3. Cirrhosis as diagnosed by
   - liver histology or
   - clinical and imaging examination (may include fibroscan).
4. Patient with Acute Decompensation of cirrhosis
5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)
6. The INR measurement has to be : $1.2 \leq INR < 2$

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.

   Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.
3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion.

4. Variceal banding or sclerosis within 4 weeks before the infusion
5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
6. Clinical evidence of Aspergilus infection.
7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion.
8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
9. Coagulation disorders defined as :
    - INR ≥ 2
    - Fibrinogen < 100 mg/dL
    - Platelets < 50.000/mm^3
10. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator.
11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.
12. MELD score > 30.
13. Previous organ transplantation and/or ongoing immunosuppressive treatments.
14. Postoperative-decompensation following hepatectomy.
15. Renal failure due to chronic kidney disease.
16. Clinically significant left-right cardiac shunt.
17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
21. Participation to any other interventional study within the last 4 weeks.
22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration are the following:

- **Transitory discontinuation:** Coagulation disorders considered as significant (INR ≥ 2, Fibrinogen < 100 mg/dL, or Platelets < 50.000/mm^3) by the PI prior to each infusion should preclude the administration of Hepastem.
- Absence of portal vein flow prior to the infusion should preclude the administration of Hepastem.
- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.

Infusions may be restarted after recovery. If planned infusions are not performed within treatment period (± 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAWAL CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).
- The exact dosage (volume) of Hepastem infused to the patient will be calculated based on the weight of the patient on the day of infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells/infusion (5 mL) for cohort 1b or $0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells/infusion (10 mL) for cohort 2.)
- As the exact volume to infused can be low (depending on the patient's weight), it is recommended to flush after the infusion physiological solution (NaCl 0,9%) to ensure that all the product is infused.

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and the syringe will

be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells per infusion). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapated accordingly. A single infusion is expected to last minimum 5 min (for 5 mL of reconstituted Hepastem).

For cohort 2a and 2b: 3 patients will receive HepaStem in a single infusion (cohort 2a) or in 2 repeated infusions one week apart (cohort 2b). The dosage of Hepastem per infusion will be $0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion).

Each infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single infusion is expected to last minimum 10 min (for 10 mL of reconstituted Hepastem).

The full procedure describing how to adapat the volume of HepaStem to be administrated to the patient's body weight is in the HepaStem Manual.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate,ICUs or standard units. Patients will be hospitalised during HepaStem treatment period to allow a continuous monitoring of the patient.

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension has to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation : the syringe has to be inversed 10 times every 3 minutes.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics,risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), and risks observed with the infusion of HepaStem in the cohort 1a, main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis or to consumption of coagulation factors and subsequent bleeding; respiratory disorder as cells first transit to the lungs; hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the risks mentioned below, there might be other, at this time, unknown risks.

5.4.1. Risk and Benefit assessment

ACLF patients or patients at high risk to develop ALCF are at high mortality risk and there is currently no specific treatment for these patients. Orthotopic liver transplantation is often not a possible option for these patients. By its potential combined effects, HepaStem could play a favourable role in restoring an immunological balance in pre-ACLF / ACLF patients, leading to a resolution of this acute event and showing improvement of organ function and transplantation free survival. The main identified risks linked to HepaStem are activation of the coagulation cascade and may lead to thrombosis (observed in UCD patients) or bleeding (observed in ACLF patients). The safety measures described below (see section 5.5) are recommended to minimize the risks of the administration of HepaStem in ACLF or pre-ACLF patients at high risk of short term mortality.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the

Before each infusion, the investigator will have to make sure the patient has the minimum criteria to receive HepaStem (see 4.4 - Criteria for study treatment discontinuation).

Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lung disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect.

Number of cells administered for the cohort 1a, 2a and 2b per infusion will be maximum 25.10^6 cells (cohort 1a) or 50.10^6 cells (cohorts 2a and 2b) and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min. The lower dose regimen will be applied before the higher one. These doses are in the very low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication and re-adjusted to the range of MSC doses given in decompensated cirrhotic and ACLF patients (See Section 1).

Furthermore, **the coagulation parameters will be closely monitored** prior and after the infusion process at 4h, 8h, 12h, 24h, 48h and 72h post infusion. (Including INR, aPTT, fibrinogen, D-Dimers, coagulation factors (pre and 24h post infusion), and TEG (optional, only if measurement can be done locally and up to investigator's judgment)

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion, and patient with risk of bleeding (defined by recent major invasive procedure, non controlled gastrointestinal hemorrhage and/or coagulation disorders) will be excluded from the study.

In case major changes in the coagulation parameters and/or clinically significant bleedings suggestive of important coagulation factors consumption occur, according to the investigator's judgement, it could be envisioned to administer coagulation factors in the form of fresh frozen plasma (FFP), coagulation factor concentrate (ie Cofact containing Factors II, VII, IX, X plus protein S and protein C), fibrinogen concentrate (ie RiaSTAP), and/or antifibrinolytics (ie tranexamic acid). (cfr. both study patients in cohort 1a responded well to treatment with FFP and/or addition of coagulation factors).

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions.

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Long Term Safety follow-up Period of 1 year. Thereafter, they will be followed-up the the Patient Registry.

Thereafter, patients will be followed in the Patient Registry.

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1

On HepaStem infusion days, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, coagulation factors, TEG (if already performed as part of the clinical routine and up to investigator's judgment) a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

The careful evaluation of these parameters will allow or not the infusion (see section 4.4 – Criteria for treatment discontinuation).

On the other days during the hospital stay, patients will be followed-up according to usual practice.

A study visit will be performed on Day 14 $\pm$ 2 days, including the evaluations listed below.

After the treatment period, study visits will be done on days 21 and 28 ($\pm$ 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 ($\pm$ 2 weeks), Month 3 ($\pm$ 2 weeks), Month 6 ($\pm$ 2 weeks), and Year 1 ($\pm$ 1 month).

Up to the 28 days visit, all SAEs will be collected. After the 28 days visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be included in the patient Registry.

6.2.1. Study assessments

- All AEs up to Day 28

- All AESI up to 1 Year.
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation),MELD score and Child Pugh will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
    - GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
    - INR
    - aPTT
    - fibrinogen
    - D-Dimers
    - TEG (optional, only if measurement can be done locally and up to investigator's judgment)
- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion.
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening. Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2), 12, 14, 21, 28 (blood testing in central lab)

- Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also on M12
- Chest x-ray at screening (if not performed during same admission) and at M12,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)
- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

<u>Thromboelastogram and thromboglobulin generation tests</u>

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min twice). The plasma will be colleted and put in cryotubes (min. 2 tubes containing each min. 500 µL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Hippocrate 10,
1200 Woluwe-Saint Lambert BELGIUM

<u>Anti-HLA antibodies</u>

Serum will be collected and send within 48 hours (ambient temperature) to :

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Hippocrate 10 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHARTS

Table 6-1 Study Flowchart

Period	**Screening Period**	**Active period**							***Long term follow-up***			
	Baseline	**Treatment Period**					**Surveillance Period**					
Time	**Maximum 7 days prior D1**	Infusion D1	D4[b]	D8[c]	D12[b]	D14[b]	**D21[b]**	**D28[b]**	**M2[d]**	**M3[d]**	**M6[d]**	**M12[e]**
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	X	«	X	X	X	X	X	X	X	X
Vital Sign	X	↔	X	↔	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	«[%]	X	«[%]	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	X	«	X	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	+	X	+	X	X	X	X	X	X	X	X
Coagulation 2 : C-Protein, S-Protein, Anti-Thrombin III	X[§]											
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen, D-Dimers, TEG@		+		+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	&		&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	«		«		X		X				
TEG, TG	X	*	X	*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	X	«	X	X	X	X				X
Chest X-Ray	©											X
Cardiac US Doppler	©	≠										X
ECG	©											
Blood culture or other fluid culture	A											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10[6] cells /kg body weight with a maximum of 25.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2a : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2b : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]		X[a]								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a) Hydrocortisone given 15-30 min before HepaStem infusion

b) ± 2 days

c) ± 2 days with at least 7 days interval without infusion

d) ± 2 weeks

e) ± 1 month

« Before infusion .

A : If already performed during same admission, results collected

% : On infusion day, platelets measurement to be performed prior and post infusion at 1h, 24h, 48h and 72h

© if not already performed during same admission; if already performed, results collected

↔ before, during, after infusion

≠ : cardiac US to be performed after infusion

@ : Optionnal, only if measurement can be done locally and up to investigator's judgment

+ : On infusion day : prior and 1h, 3h, 5h, 8h, 12h, 18h, 24h, 48h and 72h post infusion (frequency of these exams can be increased up to the investigator's judgment.)

* : Before infusion and 3h post infusion

AESI : only Adverse Event of Special Interest to be reported

TEG: Thromboelastogram, TG: Thrombin generation

& : Prior and 24h after infusion

§ : In case of deterioration of the coagulation, these measurements will be repeated.

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome

- Liver Transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possible, probable or definite.

7.1.5. Serious Adverse Drug Reactions (SADR)

A SADR is any ADR that is serious as per the above criterias.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

<u>Possibly</u>

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probably

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definitly

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

Moderate, when interfering with ADL;

Severe, when impeding ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients

enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SADR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts each with 12 hospitalized patients in total.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

The 3 first patients infused (cohort 1a) : 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage (see section 1).

The next 3 patients' cohort will receive a lower dose of HepaStem and the next 6 patient's cohorts (high dose cohort) will receive the higher dose.

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Since it is a safety study, any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

The total sample size consideration remained unchanged with a total of 12 patients

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied.

Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], , if the patient is not able to fully understand the study based on the investigator's judgment, the informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant

protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences

must “verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection”.

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator’s Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient’s further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of members, all external and independent to Promethera The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.

2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.

As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort has received HepaStem infusions (complete scheme of premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patients parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a unique administration of $0.5.10^6$ cells/kg body weight).

5. The SMC will review severe coagulation events assessed as related to HepaStem administration by the investigator.
6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- One or more biostatisticians
- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists and Promethera Biosciences members may provide additional information if additional expertise is desired, but are not members of the SMC.

The frequency of SMC meetings will depend on several factors including the rate of enrollment, completion of patients in the dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL, rena replacement or use o vasopressors for Hepatorena syndrome indication
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

Arroyo et al. 2015

11.2. CLIF ACLF GRADE

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

11.3. CLIF-C ACLF SCORE

CLIF-C ACLF = $10 \times [(0{,}33 \times \text{CLIF OF} + 0.04 \times \text{Age} + 0{,}63 \times \text{Ln}(\text{WBC}\{10^9 \text{ cells/L}\}) - 2]$

11.4. CLIF CONSORTIUM ACUTE DECOMPENSATION SCORE (CLIF-C AD)

CLIF-C AD = 10 × [(0,03 × Age {years} + 0,66 × Ln(Creatinine{mg/dL} + 1.71 × Ln(INR) + 0,88 × Ln(WBC{10^9 cells/L}) – 0,05 × Sodium {mmol/L} + 8]

Jalan et al. 2015

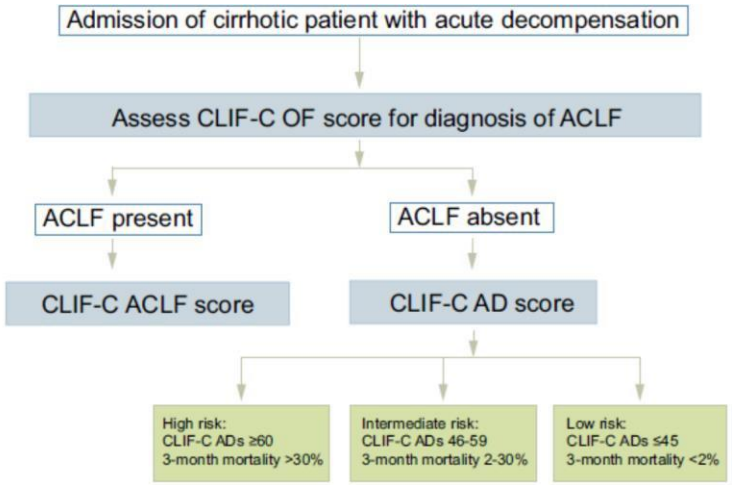

Fig. 4. Algorithm for the sequential use of the EASL-CLIF Consortium predictive scores in patients with cirrhosis admitted to hospital with acute decompensation.

11.5. MELD SCORE

MELD score is calculated using serum bilirubin, serum creatinine, and International Normalized Ratio (INR) and is given by the formula :

MELD(i) = (0.957 * ln(Serum Cr) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10 (if hemodialysis, value for Creatinine is automatically set to 4.0)

MELD Score (2016) = MELD(i) + 1.32*(137-Na) – [0.033*MELD(i)*(137-Na)]

Note: Sodium has a range of 125-137 mEq/L

The score can be calculated using online website https://www.mdcalc.com/meld-score-model-end-stage-liver-disease-12-older

11.6. CHILD PUGH SCORE

Measure	1 point	2 points	3 points
Total bilirubin, µmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8

Prothrombin time prolongation (s)	<4.0	4.0–6.0	> 6.0
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	Class	One year survival	Two year survival
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

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11.7. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 15 Feb 2018

Version Number: 4.0

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____ _____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 15 Feb 2018

Version Number: 4.0

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competent Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

Silver Ocean Ventures SAS, CEO, represented by John Tchelingerian Promethera Biosciences	Date
Etienne Sokal, Chief Scientific & Innovation Officer Promethera Biosciences	Date
Nancy Veulemans, Vice-President Clinical & Medical Affairs Promethera Biosciences	Date

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 5.0 _ 26 June 2018

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Scientific & Medical Officer John Tchelingerian, PhD – Chief Executive Officer
MONITORING & STUDY COORDINATION	Virginie Barthel, T.+32.471.784.097
SAFETY & PHARMACOVIGILANCE	pv@promethera.com **T.*+32.10.39.43.43***

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LIST OF ABBREVIATIONS

AD	Acute Decompensation
ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SADR	Serious Adverse Drug Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 5.0 – 26 Jun 2018
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Up to twenty one(21) evaluable Patients
Number of Centers	Up to 25 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts with 18 (up to 21) hospitalized patients in total 5 patients were screened in the cohort 1a on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next patients enrolled will complete each dose cohort in a step wise approach and will receive a lower dose following SAEs observed in patient 2 and patient 3 in the cohort 1a (with high dose of cells).

Twelve (up to fifteen) other patients will be enrolled in cohort 2.

3 (up to 6) patients of cohort 2 (cohort 2a) will receive twice the dose compared to cohort 1b.

3 (up to 6) patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort.

The 3 patients of the cohort 2c and 2d will receive up to 2 times the dose given in to the cohort 2b

The statistical analysis will take into consideration the different doses applied.

Study periods

The study will recruit cirrhotic patients who are hospitalized for ACLF or Acute Decompensation at risk of developing ACLF

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Patients with Acute Decompensation of cirrhosis at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining signed informed consent, the screening period may last maximum 7 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

	The study is divided in the following periods: screening period, active study period divided in treatment period and evaluation period, and long-term safety follow-up. Screening period: Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. Active study period: The active study period will last 28 days (± 2 days). The duration of the screening period plus the active period will last up to 35 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Various dose regimens of HepaStem will be given, which differ in the amount of cells per infusion and/or in the number of infusions. The 3 patients in the cohort 1a received the dose regimen of 250.10^6 cells per infusion – this represents approximately 2.9 to $3.4.10^6$ cells/kg bodyweight in the first cohort. (cohort 1a) The next three patients in cohort 1 (cohort 1b) will receive a lower dose (minimum ten times lower) in a single infusion ($0.25.10^6$ cells /kg bodyweight with a maximum of 25.10^6 cells per infusion). 3 (up to 6) patients in cohort 2 (cohort 2a will receive twice the dose of the patients in cohort 1b ($0.5.10^6$ cells/kg bodyweight with a maximum of 50.10^6 cells per infusion). 3 (up to 6) patients in cohort 2 (cohort 2b) will receive up to 2 doses of $0.5.10^6$ cells/ kg bodyweight 1 week apart ($0.5.10^6$ cells/kg bodyweight per infusion with a maximum of 50.10^6 cells per infusion). A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort.

	The 3 next patients in cohort 2 (cohort 2c) will receive twice the dose of the patients in cohort 2a (1.10^6 cells/kg bodyweight with a maximum of 100.10^6 cells per infusion). The 3 next patients in cohort 2 (cohort 2d) will receive up to 2 doses of 1.10^6 cells/ kg bodyweight 1 week apart (1.10^6 cells/kg bodyweight per infusion with a maximum of 100.10^6 cells per infusion). Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of members, all external and independent to Promethera Biosciences. As a minimum, the safety data will be reviewed by the SMC at the following timepoints : - For each cohort (1b, 2a, 2b, 2c and 2d), when the first evaluable patient has received the HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients. - When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advice on the enrolment of patients in the next cohort. If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient. More specifically, based on the patients' parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg bodyweight) instead of a single administration of $0.5.10^6$ cells/kg bodyweight). <u>Long-term safety follow-up</u>: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the safety follow-up period. After completion of this study, patients will be invited to be followed-up in the Patient long term safety follow up Registry for 5 additionnal years.
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (35 days ($\pm$ 2 days) in the screening plus active treatment period), and thereafter for an additional period of 1 year in the safety follow-up.

Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at $50x10^6$ cells/ml equivalent to $250x10^6$ cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent. In cohort 1a, 50 ml was given per infusion. For cohorts 1b, 2a, 2b, 2c and 2d, the volume of HepaStem administered will be adapted to the patient's bodyweight.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on the patient's status. The infusion rate shall not exceed 1 ml per min. For cohort 1, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion had to be respected between infusion days. The *Planned* schedule was: in cohort 1a, 250 million cells in 50 ml were administered on each infusion day, leading to a total of 1 billion cells, if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone was expected to be given 15 to 30 min before each HepaStem infusion. The *Actual* schedule is: in cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2a: 3 (up to6) patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

	For cohort 2b: 3 (Up to 6) patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation). A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort. For cohort 2c: 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2d: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).
Eligibility - Inclusion Criteria	<u>Inclusion criteria</u>: 1. Adult aged between 18 and 70 years old. 2. Signed Informed Consent. <u>N.B:</u> In case of hepatic encephalopathy, if the patient is not able to understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

	3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan). 4. Patient with Acute Decompensation of cirrhosis 5. Serum total Bilirubin ≥ 6 mg/dL (≥100 umol/L)
Eligibility - Exclusion Criteria	<u>Exclusion criteria</u>: 1. Thrombosis of the portal vein. 2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion. 3. Ongoing uncontrolled bleeding. 4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 5. Clinical evidence of Aspergillus infection. 6. Circulatory failure defined by inability to maintain a mean Blood pressure ≥ 70 despite use of vasopressors 7. Mechanical ventilation due to respiratory failure 8. Coagulation disorders defined as : • Fibrinogen < 80 mg/dL • Platelets < 40.000/mm^3 9. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator. 10. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period. 11. MELD score > 35. 12. Previous organ transplantation and/or ongoing immunosuppressive treatments. 13. Postoperative-decompensation following hepatectomy. 14. Renal failure due to chronic kidney disease. 15. Clinically significant left-right cardiac shunt. 16. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium

	bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions. 17. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. In case of suspicion of HCC, all exam should be done to confirm or not the diagnosis prior enrolment. 18. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 19. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 20. Participation to any other interventional study within the last 4 weeks. 21. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisousness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28. The relationship will be assessed based on investigator assessment, and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline. <u>Secondary Endpoints</u>: • Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Mortality ○ Liver transplantation ○ Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score, Child Pugh score. • Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Bilirubin, creatinine, INR and albumin values • Long term safety follow-up ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1

	▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation New ACLF episode will be summarized at Month 3 and Year 1
Study Assessment visits	<u>Study visits</u> During the screening and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. On HepaStem infusion days, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, TEG (if already performed as part of the clinical routine and up to investigator's judgment), coagulation factors (intrinsic and extrincsic pathway), a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day (even if HepaStem infusions are prematurily stopped). The careful evaluation of these parameters will allow or will not allow the infusion (see section 4.4 – Criteria for treatment discontinuation). On the other days during the hospital stay, patients will be followed-up according to usual practice. A study visit will be performed on Day 4, 8, 12 and 14 ± 2 days post 1st infusion, including the evaluations listed below. After the treatment period, study visits will be done on days 21 and 28 (±2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged. After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

	Up to Day 28 visit, all SAEs will be collected. After Day 28 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new AD and/or ACLF episode, AEs assessed by the investigator as possibly related to HepaStem (see Section 7.1.2).
	
	At Month 12 study visit, patients will be invited to be included in the Patient Long term follow up registry.
	
	<u>Study assessments</u>
	
	• All AEs up to Day 28
	• All AESI up to 1 Year.
	• Concomitant medication modifications up to Day 28
	• Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD score (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12,, 14, 21, 28, Months 2, 3. 6 and 12
	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
	  ○ GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
	  ○ Creatinine, Urea or BUN
	  ○ CRP
	  ○ INR, aPTT
	  ○ Serum albumin, sodium, potassium
	• Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
	  ○ INR

	○ aPTT ○ fibrinogen ○ D-Dimers ○ TEG (optional, only if measurement can be done locally and up to investigator's judgment) • Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion. • Lipase: at screening • Viral serology (HIV, HCV, HEV, HbS antigen) and Aspergilus detection: at screening (if not performed during same admission) • Urine test (Sediment, Creat, Glc, Protein, Albm): at screening • Protein C, Protein S, anti-thrombin III: at screening. In case of deterioration of the coagulation, these measurements will be repeated. • Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2b), 12, 14, 21, 28 (blood testing in central lab) • Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab) • Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab) • Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also at M12. • Chest x-ray : at screening (if not performed during same admission) and at M12, • Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected) • ECG: at screening (if not performed during same admission). • Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12. A SMC will review safety data and advise on study conduct. Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion. In case of premature withdrawal from study, an end of study visit should be perfomed if possible at the time of study withdrawal.

	In case of liver transplantation during the course of the study, a sample of the explanted liver will be collected if possible.
Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem is administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist. The 3 first patients infused (cohort 1a) received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. 2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage to a new low dose. (see section 1)). The next 3 patients will receive a lower dose of HepaStem and the next 12 (up to 15) patients (new high dose cohort) will receive the higher dose. The total sample size consideration will be a total of 18 (up to 21) patients.
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who received at least one infusion.

	All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

	The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The Report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Period	**Screening Period**	**Active period**										
	Baseline	**Treatment Period**					**Surveillance Period**		***Long term follow-up***			
Time	**Over 1-7 days prior D1**	Infusion D1	D4 ± 2 days	D8 ± 2 days	D12 ± 2 days	D14 ± 2 days	**D21** ± 2 days	**D28** ± 2 days	**M2** ± 2 weeks	**M3** ± 2 weeks	**M6** ± 2 weeks	**M12** ± 1 month
Informed Consent	X											
Eligibility criteria	X											
Demography & Medical History	X											
Physical exam	X	Xa	X	Xa	X	X	X	X	X	X	X	X
Vital Sign	X	Xb	X	Xb	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD, MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	X%	X	X%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	X%	X	X%	X	X	X	X	X	X	X	X
Lipase & Coagulation 2: C-protein, S-protein, Anti-Thrombin III	X											
Coagulation 1 : INR, aPTT	X	X+	X	X+	X	X	X	X	X	X	X	X
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen	X	X+		X+								
Coagulation 3 : D-Dimers, optional local TEG		X+		X+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	Xa		Xa		X		X				
TEG, TG	X	X*	X	X*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	Xa	X	Xa	X	X	X	X				X
Chest X-Ray	©											X
ECG	©											
Cardiac US Doppler	©	≠										X
Blood culture or other fluid culture	c											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶cells /kg body weight with a maximum of 25.10⁶cells + Hydrocortison (100mg) 15-30min prior infusion		X										
Cohort 2a & b : Infusion of 0.5.10⁶cells /kg body weight with a maximum of 50.10⁶cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2b only								
Cohort 2c & d : Infusion of 1.0.10⁶cells /kg body weight with a maximum of 100.10⁶cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2d only								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a: On infusion day: before infusion

b: On infusion day: before, during and after infusion

%: On infusion day: all parameters are measured prior infusion/platelets measurement to be performed prior and post infusion at 4h, 24h, 48h & 72h

C: Only if performed during the same admission

+: On infusion day: prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion

&: On infusion day: prior and 24h after infusion

©: if not already performed during same admission. If already performed, results collected

*: On infusion day: prior and 4h after infusion

AESI: Only Adverse Event of Special Interest to be reported

≠: cardiac US to be performed after infusion

1. BACKGROUND AND RATIONALE

1.1. CIRRHOSIS, ACUTE DECOMPENSATION AND ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Cirrhosis is a progressive chronic liver disease characterized by diffuse fibrosis, severe disruption of the intrahepatic venous flow, portal hypertension and liver failure. The course of cirrhosis is divided into two stages. Compensated cirrhosis defines the period between the onset of cirrhosis and the first major complication. During this period, which is relatively long in most patients (>10 years), symptoms are absent or minor, but liver lesions and portal pressure steadily progress. The term decompensated cirrhosis defines the period following the development of ascites (that is, the accumulation of large amounts of fluid within the peritoneal cavity), variceal haemorrhage and/or hepatic encephalopathy. This period is associated with short-term survival (3–5 years). It is increasingly evident that patients rarely die as a consequence of an end-stage irreversible destruction of the liver. Rather, in most patients, the cause of death is an acute deterioration in their chronic clinical condition promoted by a precipitating event — a syndrome termed acute-on-chronic liver failure (ACLF) (Arroyo et al. 2015).

It is of note that definitions on ACLF may differ worldwide. Given the heterogeneity and the importance of identifying patients four major societies/organisations have provided working definitions (APASL, NACSELD, WGO and EASL-CLIF). The common definition of ACLF is 'a syndrome characterised by acute decompensation of chronic liver disease associated with organ failure(s) and high short-term mortality'. According to the CLIF-ACLF definition developed based on the CANONIC study, ACLF is a recognised syndrome characterised by acute decompensation of cirrhosis associated with the failure of one or more organs and, in the more severe cases, system failure. The organs and systems most likely to fail are the liver, kidney, brain, coagulation, circulation and/or lungs. Patients have a high short term mortality of over 15 % at 28 days (Hernaez R et al, 2017). In the CANONIC study approximately 31% of patients admitted to a hospital for Acute Decompensation (AD) of cirrhosis had ACLF at admission (20%) or developed the syndrome during hospitalisation (11%). The common causes of acute decompensation of liver function included bacterial infections, alcoholic hepatitis, and gastrointestinal hemorrhages, but, in more than 40 % of patients, no precipitating event was identified (Moreau et al. 2013). Among patients with Acute Decompensation (AD), subgroups were identified as being at higher risk of progressing to full blown ACLF and thus at higher mortality risk (Arroyo et al. 2015).
Different grading/scoring systems have been developed in order to better determine prognosis and effectiveness of intervention and care. (Hernaez R et al, 2017).

In daily practice, MELD and Child Pugh scores are still strongly relied on to guide clinical care.

The Model for End-Stage Liver Disease, or MELD, is a scoring system for assessing the severity of chronic liver disease. This score is used by the United Network for Organ Sharing (UNOS) and Eurotransplant for prioritizing allocation of liver transplants. New MELD uses the patient's values for serum bilirubin, serum creatinine, sodium and the international normalized ratio for prothrombin time (INR) to predict survival.

Mortality and MELD score are linearly correlated amongst patients with end-stage liver disease listed for OLT with 3-month mortality estimated to be 4%, 27%, 76%, 83%, and 100% for MELD scores of <10, 10–19, 20–29, 30–39, and 40 or more respectively.

The Child–Pugh score is used in clinical practice to assess the prognosis of chronic liver disease, mainly cirrhosis. It was previously used for prioritizing allocation of liver transplants. The score employs five clinical measures of liver disease: total bilirubin, serum albumin, prothrombin time, ascites and hepatic encephalopathy. Each measure is scored 1–3, with 3 indicating most severe derangement. This leads to three Classes with one year overal survival of 100% for Class A, 81% for class B and 35% for class C. (see 0)

ACLF has been defined by the CLIF research consortium into four grades based on retrospectively fitting data on severity linked to mortality score (Moreau et al. 2013) (Table 1-1 and Table 1-2)

- ACLF grade 0 concerns 69.1 % of patients admitted to hospital with acute decompensation. The group is defined as no organ failure, single "non kidney" organ failure (ie, single failure of the liver, coagulation, circulation, or respiration) who had a serum creatinine level < 1.5 mg/dL and no hepatic encephalopathy, or as single cerebral failure with a serum creatinine level < 1.5 mg/dL. These patients have a 28-day and 90-day mortality rate of 4.7% and 14% respectively.
- ACLF grade 1 concerns 15.8 % of patients admitted to hospital with acute decompensation. The group is defined as single kidney failure or single non-kidney organ failure with an organ dysfunction (kidney or brain) and has a 28-day mortality rate of 23 %.
- Patients with ACLF grade 2, defined as two failing organs (10.9 % of patients admitted to hospital with acute decompensation) has an intermediate prognosis (28-day mortality rate of 31%).
- Finally, ACLF grade 3, defined as three or more organ failures (4.4 % of patients admitted to hospital with acute decompensation) has extremely high mortality rates, reaching 75 % after 28 days.

Among patients hospitalised with acute decompensation (AD) (pre ACLF according to the CLIF criteria but ACLF according to other classification systems), an analysis revealed five independent variables including age, serum sodium, white cell count, creatinine and INR as useful for defining a scoring system. The high-risk group (CLIF-C AD score > 60) and intermediate risk group (CLIF-C AD score 46-59) respectively have a 3-month mortality of over 30 % and between 2-30 %. The low risk AD group has a 3-month mortality below 2 % (Arroyo et al. 2015).

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years.The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

Cirrhotic patients with acute decompensation can only receive supportive treatments, such as antibiotics in case of infection, lactulose in case of encephalopathy, terlipressin and albumin in case of hepatorenal syndrome. However, at this moment, there are no treatments available to stop the inflammatory cascade often accompanying the acute decompensation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion,

which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 , renal replacement or use of vasopressors for Hepatorenal syndrome indication
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may cause necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver transplant rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

Conclusion on patient population: Based on this information, Promethera Biosciences proposes that the patient population is defined as cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 µmol/L) and MELD < or = 35.

Patients should have coagulation parameters within the ranges below:

- Fibrinogen ≥ 80 mg/ dL
- Platelets ≥ 40.000/mm3

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidence supports the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properties of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells (similar cells to HHALPC) were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013).

Later, the technology of large-scale cell production was transferred to Promethera Biosciences where clinical batches of HHALPC are produced in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs).

Toxicology *in-vitro or in-vivo* studies aiming to demonstrate the safety, tolerability and tumorigenicity aspect of HepaStem were conducted. *In vivo* studies were performed in rats and mice. They included one study to assess the safety of the intravenous mode of administration. Two studies specifically assessed the risk of tumor formation as this risk has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. An *in vitro* study the pro-coagulant activity of HepaStem was confirmed (Please refer to the IB for more details).

In addition, *invitro* studies show that HepaStem cells express variable immunomodulatory surface markers of interest and have immunomodulatory functional effects: HepaStem inhibits the proliferation of activated T-lymphocytes and blocks the maturation of monocytes (see Section 1.2.6). Furthermore, 6 *in-vivo* studies were conducted with HepaStem evaluating the immunomodulatory properties using the IV route of administration and mainly doses of 12.5 10^6 cells/kg. No safety signal was detected based on these *in vivo* studies.

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg) (4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did

undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. These data laid the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

Based on literature data and Promethera experiences, it can be concluded that liver-derived MSCs, including HepaStem have a pro-coagulant activity. This pro-coagulant activity is also expressed by other MSCs. The pro-coagulant activity might be linked to tissue factor expression, an activator of the coagulation cascade. The procoagulant effect could be modulated by the concomitant administration of bivaluridin during HepaStem infusion in UCD clinical trials in order to prevent, mainly, anticipated thrombotic events. Very high cell doses have been administered intra-portally in the UCD studies in which thrombotic events only occurred at high doses (range: 115 million to 4,1 billion total cells were administered in the portal vein as a split dose in 1 to 10 infusions spread over 1 to 4 consecutive days). Bivaluridin will not be used in the ACLF clinical study as its use has not been validated for late stage cirrhotic patients. Contrary to patients with urea-cycle disorders, coagulation disturbances are common in the late stage chronic cirrhosis population and are linked to liver insufficiency. (see 1.2.5)

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

In a first-in man cohort conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells (ADHLSC, similar cells to HHALPC) infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated this cell infusion well, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells

repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, spleen, right ankle, and spine, and had disappeared from the lungs. This is in line with the bio-distribution of another type of MSCs administrated in patients (BM-derived MSCs) that demonstrate a similar bio-distribution, with a first pass through the lung; within 24 hours, cells are mainly found in liver, spleen, kidneys and other inflamed areas, by 48 hours, more pronounced presence in the liver is observed. (NDS dossier remestemcel-L, Health Canada).

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For example, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012).

In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

It is clear from consultation of literature publications on cell therapy administration in advanced liver disease including decompensated liver cirrhosis and ACLF that doses of cell therapy protocols tended to be lower as compared to the normal range administered to patients in other immune-modulatory or anti-inflammatory protocols.

The doses and regimens administered to treat patients with chronic liver diseases, range from 0.03 over 0.5 to 1 million MSC cells/kg bodyweight. Different regimens were applied with repeated dosing up to 3 times for the lowest doses (0.03; 0,05 and 0,5 million cells/kg BW repeated 3 times). Most protocols administered the cell infusion intravenously although also other routes of administration were investigated such as intra-splenic, hepatic artery, intrahepatic, intra-lesional route of administration or central venous catheter into the femoral vein. (Berardis et al. 2015)

Based on the literature review, MSC administration is considered to be safe due to the lack of reports of significant adverse effects in the above studies, although a marked heterogeneity was observed among studies with regard to injection dose, frequency of injection, cell source, delivery route and study design. Most of these early studies reported improvements in liver function, ascites and encephalopathy.

In the first cohort of 3 patients in the HEP001 study the lowest dose (12.5×10^6 cells/kg) of the range of doses administered safely in previous studies of HepaStem in urea cycle disorder (UCD) and Crigler Najar pediatric patients was used to determine the dose in the HEP101 protocol. The (low) dose proposed (250

million cells/ infusion; ie. 3.5 10^6 cells/kg BW/infusion) was a reduction of 4x of the lowest dose tested previously (in the HEP001 protocol) and it was thought that it could be safely administered in cirrhotic patients. Additionally, the number of cells administered per infusion would be limited, similar to MSC doses given in immune mediated inflammatory diseases. In retrospect, it was clear that adaptation to the dose level similar to other MSCs given in immune-mediated inflammatory diseases was inadequate and did not take the specific case of severely ill chronic cirrhotic patients with acute decompensation into account.

Therefore, it seems that using a careful approach starting with doses commonly used in reported studies of decompensated cirrhosis and ACLF patients and published as being safe, appears to be an acceptable approach. Also, dose escalation to a maximum of 1.0 million cells/kg BW per infusion should be feasible based on a repetitive dosing schedule. In case of repetitive dosing, the doses will be given weekly, which allows time in case of fibrinolysis for the parameters to be corrected and return to normal. Pre-clinical immunomodulatory data of liver-derived progenitor cells

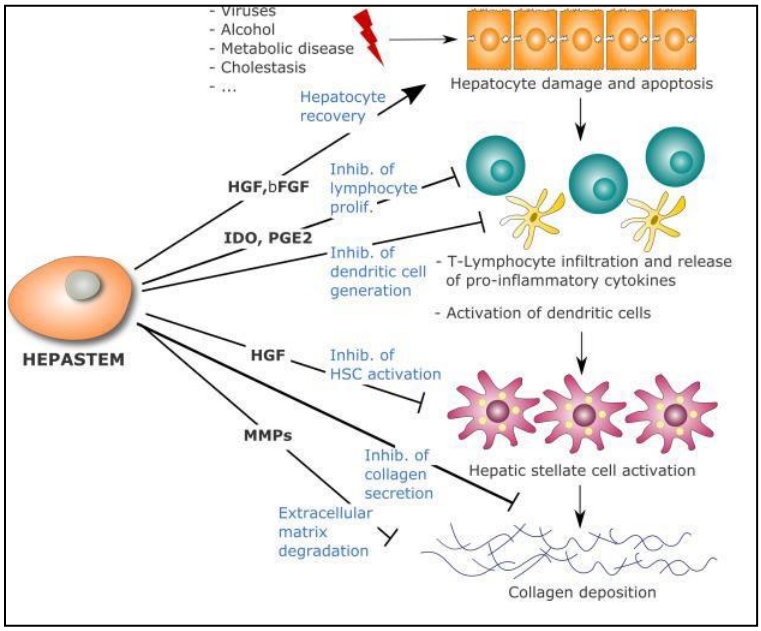
The first transcriptomics and secretomics tests performed on liver-derived progenitor cells (similar cells to HHALPC) grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (similar cells to HHALPC) (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature

dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models presents important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.6. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing to the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic

hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.7. Expected Benefits of HepaStem

Proposed mechanisms of action: after intravenous administration of HepaStem, cells are expected to circulate into the blood network where they can exert a systemic immunomodulatory action. At the same time, they have a main homing into the liver where they can thus also exert some important local immunomodulatory effects. They are expected to play their immunomodulatory roles through direct cell-to-cell interaction and through paracrine effects via the various cytokines, chemokines, MMPs and growth factors they may secrete. HepaStem could affect monocytes and DC recruitment, differentiation, maturation and function through cell contacts or paracrine signalling. HepaStem could also alter the proliferation and activation of T-lymphocytes that are another dysregulated cell type of the immune system in ACLF. In addition to modulate the behaviour of immune cells, HepaStem could modulate the proliferation and activation of hepatocytes and hepatic stellate cells and thus their secretory profiles, helping in this way the liver function recovery. The current *in vitro* and *in vivo* data, based on the scientific literature, and sponsor *in vitro* results, support all these potential immunomodulatory effects of HepaStem in ACLF patients.

Proposed clinical significant benefit: by these combined effects, HepaStem could play a favourable role in restoring an immunological balance in ACLF patients or patients at risk of ACLF, improving organ failure scores, improving clinical status, possibly leading to a resolution of this acute event and demonstrating improvement of transplantation free survival.

Considering the unmet medical need: i. the emergency to treat cirrhotic patients with Acute Decompensation (pre-ACLF or ACLF) due to the high mortality rate; ii. the shortage of healthy donors and the need of livers in the context of liver transplantation; iii. Concerns raised recently regarding artificial liver support; and iv. the mechanism of action of HepaStem, we can say that all these factors are in favour of a promising favourable benefit/risk balance for HepaStem. The exact profile of which patients will benefit most is under investigation, and also subject of this safety study.

1.2.8. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts with 18 (up to 21) hospitalized patients in total. The study will include patients with acute decompensation of cirrhosis and with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 35,excluding patients with circulatory, respiratory failure or severe coagulations disorders. It is planned to have a first group of 6 patients (cohort 1) being administered with the low dose.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (±2 days); at least a 2-day interval without infusion was respected between infusion days.

On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 patients experienced an episode of severe bleeding. Therefore, it has been decided to reduce the dose in the low dose cohort to $0.25.10^6$ cells/kg bodyweight with a maximum of 25.10^6 cells in a single infusion. A reduction of minimum 10 times the dose previously used.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

Once this has been proven safe, a second group of up to 6 patients (cohort 2a) will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: up to 6 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort.

For cohort 2c: 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2d: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).

HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period.

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 12 patients, appears to be an acceptable approach in patients with ACLF or with acute decompensation at risk of developing ACLF for whom no specific therapeutic or curative treatment exist. (See section 1.1)

HepaStem administration will be started rapidly after hospitalisation and will be completed within 1 day (cohort 1b, 2a, 2c) or within 1 week (cohort 2b, 2d). This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of patients with or at risk of developing ACLF. As ACLF and/or Acute Decompensation of cirrhosis is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up up to 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining the design of future efficacy clinical studies.

Study population

The patient population is defined by cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 35 (see section 1.1).

Patients should have coagulation parameters within the ranges below:

- Fibrinogen ≥ 80 mg/ dL
- Platelets ≥ 40.000/mm3

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort 1a - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 14 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 28 million cells/kg (assuming a patient

weight of 70 kg). The first selected dose (250 million cells per infusion) is close to MSC doses given in other trials for immune-mediated inflammatory diseases (Section 1.2.5), therefore, it was expected to show a similar safety and efficacy profile. It corresponded also to the high dose of liver-dirived progenitor cells (similar cells to HHALPC) administered via IV to the heamophila patient (see 1.2.4).

Due to the severe bleeding that occurred in 2 of the 3 patients that received 250 10^6 cells (50 mL of HepaStem) per infusion, the next selected dose (low dose cohort 1b) will be reduced to 0.25.10^6cells/kg bodyweight (with a maximum of 25.10^6 cells per infusion) administered in a single infusion (at least a 10x reduction of the dose administered in cohort 1a).

The second selected dose represents a two-fold increase from the dose in cohort 1b (0.5.10^6cells/kg bodyweight (with a maximum of 50.10^6 cells per infusion)

The third and fourth selected dose represents a two-fold increase from the cohort 2a and 2b. These dose still in the range of doses reported for MSCs and more in the range of doses administered in the specific case of severely ill chronic liver disease patients with ACLF and acute decompensation of cirrhosis. (see1.2.5)). (for additional information, please refer to the rationale for changes dated 28June2018).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 IU/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damage nor any respiratory symptoms, before homing mainly to the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF, it also can allow repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs also have a procoagulant activity comparable to liver-derived progenitor cells (similar cells to HHALPC) (Stephenne et al. 2012), nevertheless literature reports show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015).

However, liver failure results in a state of “rebalanced hemostasis” marked by a decrease in both pro-coagulation and anticoagulation factors. Patients with severe liver disease are not auto-anticoagulated. In essence, patients with severe liver disease, acute and/or chronic, have a tenuous rebalanced hemostasis that is easily perturbed by various disease states and concomitant medications and invasive procedures. Bleeding events including severe forms are common in these end-stage liver disease patients. The events of epistaxis and bleeding from puncture sites that occurred in 2 patients in cohort 1a (in retrospect a high dose in late stage cirrhotic patients), have been recognised in the literature as case reports. It was also stated that epistaxis as an overlooked cause of massive haematemesis in cirrhosis should be added to the list of upper GI bleeding.(Johal et al 2003). Hence, cirrhotic patients including ACLF patients are at increased risk of bleeding or thrombosis. Therefore dose reduction from normal ranges applied in other immune-modulatory diseases, modification of inclusion criteria and increased surveillance of liver and coagualation parameters is indicated.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of different regimens of HepaStem in cirrhotic patients presenting with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of different dose regimens of HepaStem given in subsequent cohorts with 8(up to 21) hospitalized patients in total.

5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next patients enrolled will complete each dose cohort in a step wise approach and will receive a lower dose following SAEs observed in patient 2 and patient 3 in the cohort 1a (with high dose of cells).

Twelve (Up to fifteen) other patients will be enrolled in cohort 2.

3 (up to 6) patients of the cohort 2 (cohort 2a) will receive twice the dose compared to the cohort 1b.

3 (up to 6) patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort.

The 3 patients of the cohort 2c and 2d will receive up to 2 times the dose given in to the cohort 2b.

The study will recruit patients who are hospitalized for Acute Decompensation of cirrhosis and/or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

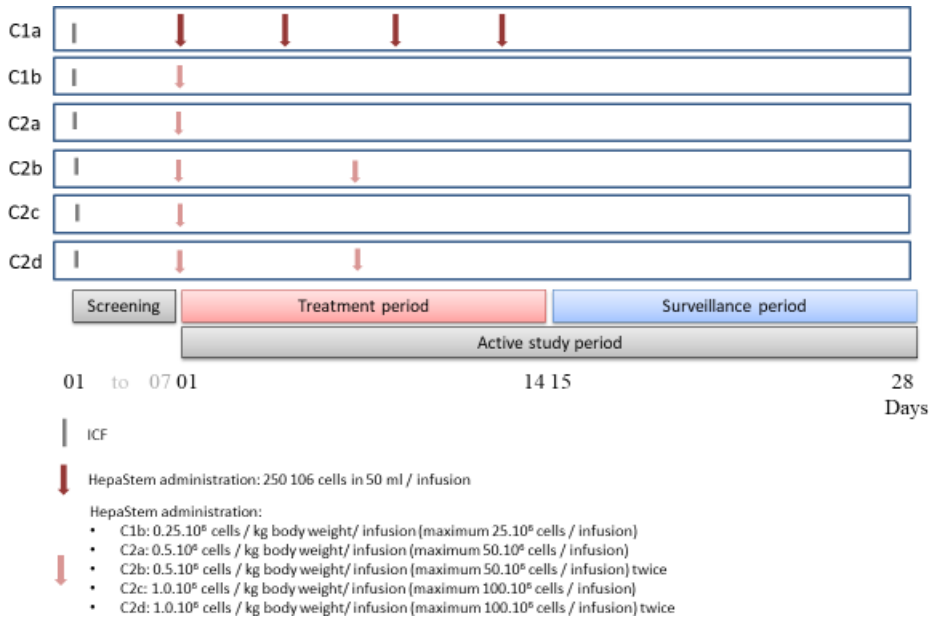
Screening period: Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days ($\pm$ 2 days). The duration of the screening period plus the active period will last up to 35 days ($\pm$ 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



Planned schedule:

For cohort 1a, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day intervalwithout infusion must be respected between infusion days.

In cohort 1a, 250 million cells in 50 ml were administrated on each infusion day, leading to a total of 1 billion cells if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion.

Actual schedule:

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

Planned schedule:

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

For cohort 2a: 3 (up to 6) patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 (up to 6) patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort.

For cohort 2c: 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2d: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).

Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of external members and Promethera members (See Section 9.13):

As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a, 2b, 2c and 2d), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The

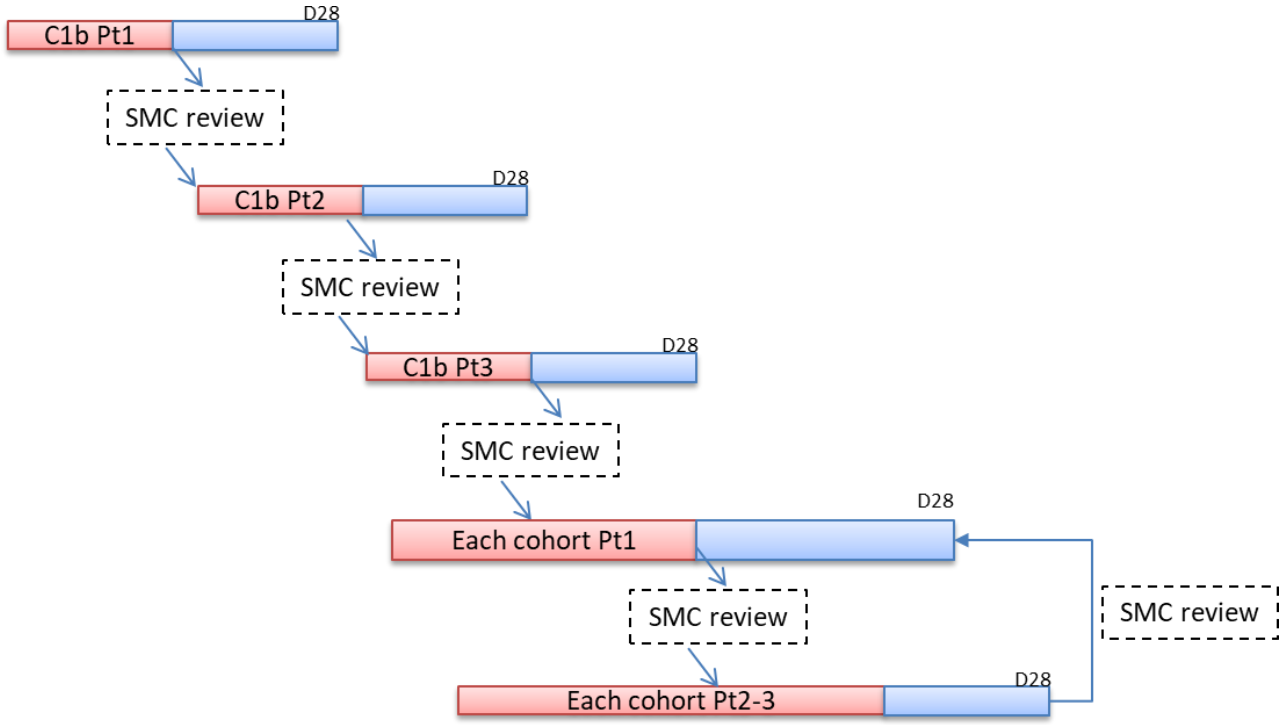
SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.

- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patients parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a single administration of $0.5.10^6$ cells/kg body weight).

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring committee



The study assessments are described in Section 6.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the safety follow-up period.

After completion of this study, patients will be followed-up in the Patient Long term Follow up Registry for 5 years.

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
  - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation
- New ACLF episode will be sumamrized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in up to 25 centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (35 days ($\pm$2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Cirrhotic patients with Acute Decompensation at risk of developing ACLF at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining signed informed consent, the screening period may last maximum 7 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 years old.
2. Signed Informed Consent.
   N.B: In case of hepatic encephalopathy, if the patient is not able to understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.
3. Cirrhosis as diagnosed by
   - liver histology or
   - clinical and imaging examination (may include fibroscan).
4. Patient with Acute Decompensation of cirrhosis
5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Thrombosis of the portal vein.
2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.

   Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.
3. Ongoing uncontrolled bleeding.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.

5. Clinical evidence of Aspergilus infection.
6. Circulatory failure defined by inability to maintain a mean Blood pressure ≥ 70 despite use of vasopressors
7. Mechanical ventilation due to respiratory failure.
8. Coagulation disorders defined as :
    - Fibrinogen < 80 mg/dL
    - Platelets < 40.0000/mm^3
9. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator.
10. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.
11. MELD score > 35.
12. Previous organ transplantation and/or ongoing immunosuppressive treatments.
13. Postoperative-decompensation following hepatectomy.
14. Renal failure due to chronic kidney disease.
15. Clinically significant left-right cardiac shunt.
16. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
17. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. In case of suspicion of HCC, all exam should be done to confirm or not the diagnosis prior enrolment.
18. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
19. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
20. Participation to any other interventional study within the last 4 weeks.
21. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration are the following:

- **Transitory discontinuation:** Coagulation disorders considered as significant (Fibrinogen <80 mg/dL, or Platelets < 40.000/mm^3) by the PI prior to each infusion should preclude the administration of Hepastem.

- Absence of portal vein flow that are in favor of a thrombosis of the portal vein prior to the infusion should preclude the administration of Hepastem.
- Transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.

Infusions may be restarted after recovery. If planned infusions are not performed within treatment period ($\pm$ 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAWAL CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).
- The exact dosage (volume) of Hepastem infused to the patient will be calculated based on the weight of the patient on the day of infusion (0.25.10^6 cells per kg bodyweight with a maximum of 25.10^6 cells/infusion (5 mL) for cohort 1b,0.5.10^6 or 1.0.10^6.cells per kg bodyweight with a maximum of 50.10^6 or 100. 10^6 cells/infusion (10 mL or 20mL) for cohort 2.)
- As the exact volume to infused can be low (depending on the patient's weight), it is recommended to flush after the infusion physiological solution (NaCl 0,9%) to ensure that all the product is infused.

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and the syringe will

be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells per infusion). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapated accordingly. A single infusion is expected to last minimum 5 min (for 5 mL of reconstituted Hepastem).

For cohort 2a and 2b: 3 (up to 6) patients will receive HepaStem in a single infusion (cohort 2a) or in 2 repeated infusions one week apart (cohort 2b). The dosage of Hepastem per infusion will be $0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion).

For cohort 2c and 2d: 3 patients will receive HepaStem in a single infusion (cohort 2c) or in 2 repeated infusions one week apart (cohort 2d). The dosage of Hepastem per infusion will be $1.0.10^6$ cells per kg body weight with a maximum of 100.10^6 cells per infusion).

Each infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single infusion is expected to last maximum 20 min (for 20 mL of reconstituted Hepastem).

The full procedure describing how to adapat the volume of HepaStem to be administrated to the patient's body weight is in the HepaStem Manual.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate, ICUs or standard units. Patients will be hospitalised during HepaStem treatment period to allow a continuous monitoring of the patient.

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension has to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation : the syringe has to be inversed 10 times every 3 minutes.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics, risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), and risks observed with the infusion of HepaStem in the cohort 1a, main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis or to consumption of coagulation factors and subsequent bleeding; respiratory disorder as cells first transit to the lungs; hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the risks mentioned below, there might be other, at this time, unknown risks.

5.4.1. Risk and Benefit assessment

ACLF patients or patients at high risk to develop ALCF are at high mortality risk and there is currently no specific treatment for these patients. Orthotopic liver transplantation is often not a possible option for these patients. By its potential combined effects, HepaStem could play a favourable role in restoring an immunological balance in pre-ACLF / ACLF patients, leading to a resolution of this acute event and showing improvement of organ function and transplantation free survival. The main identified risks linked to HepaStem are activation of the coagulation cascade and may lead to thrombosis (observed in UCD patients) or bleeding (observed in ACLF patients). The safety measures described below (see section 5.5) are recommended to minimize the risks of the administration of HepaStem in ACLF or pre-ACLF patients at high risk of short term mortality.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the

Before each infusion, the investigator will have to make sure the patient has the minimum criteria to receive HepaStem (see 4.4 - Criteria for study treatment discontinuation).

Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lung disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect.

Number of cells administered for the cohort 1a, 2a,2b, 2c and 2d per infusion will be maximum 25.10^6 cells (cohort 1a),50.10^6 cells (cohorts 2a and 2b) or 100.10^6 cells (cohorts 2c and 2d) and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min. The lower dose regimen will be applied before the higher one. These doses are in the very low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication and re-adjusted to the range of MSC doses given in decompensated cirrhotic and ACLF patients (See Section 1).

Furthermore, **the coagulation parameters will be closely monitored** prior and after the infusion process at 4h, 8h, 12h, 24h, 48h and 72h post infusion. (Including INR, aPTT, fibrinogen, D-Dimers, coagulation factors (pre and 24h post infusion), and TEG (optional, only if measurement can be done locally and up to investigator's judgment)

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion, and patient with risk of bleeding (defined by recent major invasive procedure, non controlled gastrointestinal hemorrhage and/or coagulation disorders) will be excluded from the study.

In case major changes in the coagulation parameters and/or clinically significant bleedings suggestive of important coagulation factors consumption occur, according to the investigator's judgement, it could be envisioned to administer coagulation factors in the form of fresh frozen plasma (FFP), fibrinogen concentrate (ie RiaSTAP), and/or antifibrinolytics (ie tranexamic acid). (cfr. both study patients in cohort 1a responded well to treatment with FFP and/or addition of coagulation factors).

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions.

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Safety follow-up Period of 1 year.

Thereafter, patients will be invited to be followed in the Patient long term safety follow up Registry for 5 additionnal years.5.6. Concomitant treatments

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1

On HepaStem infusion days, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, coagulation factors, TEG (if already performed as part of the clinical routine and up to investigator's judgment) a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

The careful evaluation of these parameters will allow or not the infusion (see section 4.4 – Criteria for treatment discontinuation).

On the other days during the hospital stay, patients will be followed-up according to usual practice.

A study visit will be performed on Day 14 $\pm$ 2 days, including the evaluations listed below.

After the treatment period, study visits will be done on days 21 and 28 ($\pm$ 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 ($\pm$ 2 weeks), Month 3 ($\pm$ 2 weeks), Month 6 ($\pm$ 2 weeks), and Year 1 ($\pm$ 1 month).

Up to the 28 days visit, all SAEs will be collected. After the 28 days visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be included in the patient long term safety follow up Registry for 5 additionnal years.6.2.1. Study assessments

- All AEs up to Day 28

- All AESI up to 1 Year.
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation),MELD score and Child Pugh will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
    - GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
    - INR
    - aPTT
    - fibrinogen
    - D-Dimers
    - TEG (optional, only if measurement can be done locally and up to investigator's judgment)
- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion.
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening. Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2), 12, 14, 21, 28 (blood testing in central lab)

- Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also on M12
- Chest x-ray at screening (if not performed during same admission) and at M12,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)
- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.1. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

<u>Thromboelastogram and thromboglobulin generation tests</u>

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min twice). The plasma will be colleted and put in cryotubes (min. 2 tubes containing each min. 500 µL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Mounier 53,
1200 Woluwe-Saint Lambert BELGIUM

<u>Anti-HLA antibodies</u>

Serum will be collected and send within 48 hours (ambient temperature) to :

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Mounier 53 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHARTS

Table 6-1 Study Flowchart

Period	**Screening Period**	**Active period**							*Long term follow-up*			
	Baseline	**Treatment Period**					**Surveillance Period**					
Time	**Over 1-7 days prior D1**	Infusion D1	D4 ± 2 days	D8 ± 2 days	D12 ± 2 days	D14 ± 2 days	**D21** ± 2 days	**D28** ± 2 days	**M2** ± 2 weeks	**M3** ± 2 weeks	**M6** ± 2 weeks	**M12** ± 1 month
Informed Consent	X											
Eligibility criteria	X											
Demography & Medical History	X											
Physical exam	X	Xa	X	Xa	X	X	X	X	X	X	X	X
Vital Sign	X	Xb	X	Xb	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD, MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	X%	X	X%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	X%	X	X%	X	X	X	X	X	X	X	X
Lipase & Coagulation 2: C-protein, S-protein, Anti-Thrombin III	X											
Coagulation 1 : INR, aPTT	X	X+	X	X+	X	X	X	X	X	X	X	X
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen	X	X+		X+								
Coagulation 3 : D-Dimers, optional local TEG		X+		X+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	Xa		Xa		X		X				
TEG, TG	X	X*	X	X*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	Xa	X	Xa	X	X	X	X				X
Chest X-Ray	©											X
ECG	©											
Cardiac US Doppler	©	≠										X
Blood culture or other fluid culture	c											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶ cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X										
Cohort 2a & b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2b only								
Cohort 2c & d : Infusion of 1.0.10⁶ cells /kg body weight with a maximum of 100.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2d only								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a: On infusion day: before infusion	b: On infusion day: before, during and after infusion
%: On infusion day: all parameters are measured prior infusion/platelets measurement to be performed prior and post infusion at 4h, 24h, 48h & 72h	C: Only if performed during the same admission
+: On infusion day: prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion	&: On infusion day: prior and 24h after infusion
©: if not already performed during same admission. If already performed, results collected	*: On infusion day: prior and 4h after infusion
AESI: Only Adverse Event of Special Interest to be reported	≠: cardiac US to be performed after infusion

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome

- Liver Transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possible, probable or definite.

7.1.5. Serious Adverse Drug Reactions (SADR)

A SADR is any ADR that is serious as per the above criterias.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

<u>Possibly</u>

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probably

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definitly

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

Moderate, when interfering with ADL;

Severe, when impeding ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients

enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SADR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts each with 18 (up to 21) hospitalized patients in total.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

The 3 first patients infused (cohort 1a) : 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage (see section 1).

The next 3 patients' cohort will receive a lower dose of HepaStem and the next 12 (up to 15)patient's cohorts (new high dose cohort) will receive the higher dose.

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Since it is a safety study, any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

The total sample size consideration will be a total of 18 (up to 21) patients.

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied.

Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 days active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 days active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], if the patient is not able to understand the study based on the investigator's judgment, the informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant

protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences

must “verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection”.

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator’s Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient’s further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of members, all external and independent to Promethera.The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.

2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.

As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a,2b, 2c and 2d), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme of premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patient's parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a unique administration of $0.5.10^6$ cells/kg body weight).

5. The SMC will review severe coagulation events assessed as related to HepaStem administration by the investigator.
6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- One or more biostatisticians

- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists and Promethera Biosciences members may provide additional information if additional expertise is desired, but are not members of the SMC.

The frequency of SMC meetings will depend on several factors including the rate of enrollment, completion of patients in the dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL, rena replacement or use o vasopressors for Hepatorena syndrome indication
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	 >300 >357	 ≤300 - > 200 >214- ≤357	 ≤200 ≤214

Arroyo et al. 2015

11.2. CLIF ACLF GRADE

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

11.3. CLIF-C ACLF SCORE

CLIF-C ACLF = $10 \times [(0{,}33 \times \text{CLIF OF} + 0.04 \times \text{Age} + 0{,}63 \times \text{Ln}(\text{WBC}\{10^9 \text{ cells/L}\}) - 2]$

11.4. CLIF CONSORTIUM ACUTE DECOMPENSATION SCORE (CLIF-C AD)

CLIF-C AD = 10 × [(0,03 × Age {years} + 0,66 × Ln(Creatinine{mg/dL} + 1.71 × Ln(INR) + 0,88 × Ln(WBC{10^9 cells/L}) – 0,05 × Sodium {mmol/L} + 8]

Jalan et al. 2015

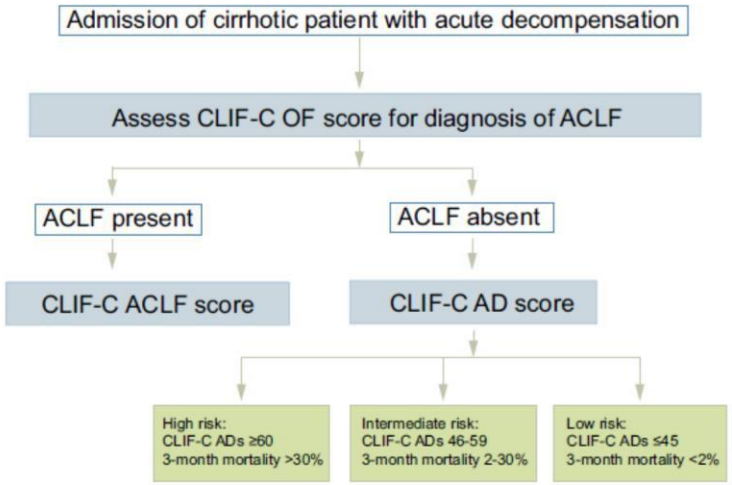

Fig. 4. Algorithm for the sequential use of the EASL-CLIF Consortium predictive scores in patients with cirrhosis admitted to hospital with acute decompensation.

11.5. MELD SCORE

MELD score is calculated using serum bilirubin, serum creatinine, and International Normalized Ratio (INR) and is given by the formula :

MELD(i) = (0.957 * ln(Serum Cr) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10 (if hemodialysis, value for Creatinine is automatically set to 4.0)

MELD Score (2016) = MELD(i) + 1.32*(137-Na) – [0.033*MELD(i)*(137-Na)]

Note: Sodium has a range of 125-137 mEq/L

The score can be calculated using online website https://www.mdcalc.com/meld-score-model-end-stage-liver-disease-12-older

11.6. CHILD PUGH SCORE

Measure	1 point	2 points	3 points
Total bilirubin, µmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8

Prothrombin time prolongation (s)	<4.0	4.0–6.0	> 6.0
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	Class	One year survival	Two year survival
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

Singal AK, Kamath PS. Model for End-stage Liver Disease. J Clin Exp Hepatol. 2013 Mar;3(1):50-60. Review.

11.7. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 26 June 2018

Version Number: 5.0

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____ _____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 26-June 2018

Version Number: 5.0

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competent Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

Silver Ocean Ventures SAS, CEO, represented by John Tchelingerian Promethera Biosciences	Date
Etienne Sokal, Chief Scientific & Medical Officer Promethera Biosciences	Date

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 5.1 – 26 June 2018

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Scientific & Medical Officer John Tchelingerian, PhD – Chief Executive Officer
MONITORING & STUDY COORDINATION	Virginie Barthel, T.+32.471.784.097
SAFETY & PHARMACOVIGILANCE	pv@promethera.com **T.*+32.10.39.43.43***

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LIST OF ABBREVIATIONS

AD	Acute Decompensation
ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SADR	Serious Adverse Drug Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 5.1 - 26 June 2018
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Up to twenty-one (21) evaluable Patients
Number of Centers	Up to 25 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts with 18 (up to 21) hospitalized patients in total 5 patients were screened in the cohort 1a, on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 ce
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 ce
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 ce

The next patients enrolled will complete each dose cohort in a step wise approach and will receive a lower dose following SAEs observed in patient 2 and patient 3 in the cohort 1a (with high dose of cells)

Twelve (up to fifteen) other patients will be enrolled in cohort 2.

3 (up to 6) patients of cohort 2 (cohort 2a) will receive twice the dose compared to cohort 1b.

3 (up to 6) patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort.

The 3 patients of the cohort 2c and 2d will receive up to 2 times the dose given in to the cohort 2b

The statistical analysis will take into consideration the different doses applied.

Study periods

The study will recruit cirrhotic patients who are hospitalized for ACLF or Acute Decompensation at risk of developing ACLF

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Patients with Acute Decompensation of cirrhosis at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last maximum 7 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

	The study is divided in the following periods: screening period, active study period divided in treatment period and evaluation period, and long-term safety follow-up. Screening period: Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. Active study period: The active study period will last 28 days (± 2 days). The duration of the screening period plus the active period will last up to 35 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Various dose regimens of HepaStem will be given, which differ in the amount of cells per infusion and/or in the number of infusions. The 3 first patients received the dose regimen of 250.10^6 cells per infusion – this represents approximately 2.9 to $3.4.10^6$ cells/kg bodyweight in the first cohort. (cohort 1a) The next three patients in cohort 1 (cohort 1b) will receive a lower dose (minimum ten times lower) in a single infusion ($0.25.10^6$ cells /kg bodyweight with a maximum of 25.10^6 cells per infusion). 3 (up to 6) patients in cohort 2 (cohort 2a) will receive twice the dose of the patients in cohort 1b ($0.5.10^6$ cells/kg bodyweight with a maximum of 50.10^6 cells per infusion). 3 (up to 6) patients in cohort 2 (cohort 2b) will receive up to 2 doses of $0.5.10^6$ cells/ kg bodyweight 1 week apart ($0.5.10^6$ cells/kg bodyweight per infusion with a maximum of 50.10^6 cells per infusion). A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort. The 3 next patients in cohort 2 (cohort 2c) will receive twice the dose of the patients in cohort 2a (1.10^6 cells/kg bodyweight with a maximum of 100.10^6 cells per infusion). The 3 next patients in cohort 2 (cohort 2d) will receive up to 2 doses of 1.10^6 cells/ kg bodyweight 1 week apart (1.10^6 cells/kg bodyweight per infusion with a maximum of 100.10^6 cells per infusion).

	Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of members, all external and independant to Promethera Biosciences. As a minimum, the safety data will be reviewed by the SMC at the following timepoints : - For each cohort (1b, 2a,2b, 2c and 2d), when the first evaluable patient has received the HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients. - For the first cohort (1b), when the second evaluable patient has received HepaStem infusion (complete scheme or premature stop), the patient data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients. - When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patients in the next cohort. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized. If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient. More specifically, based on the patients' parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg bodyweight) instead of a single administration of $0.5.10^6$ cells/kg bodyweight). Furthermore, the 3 patients of each cohort will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete sheme or premature stop). <u>Long-term safety follow-up</u>: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the safety follow-up period. After completion of this study, patients will be invited to be followed-up in the Patient long-term safety follow-up Registry for 5 additionnal years.

Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (35 days (± 2 days) in the screening plus active treatment period), and thereafter for an additional period of 1 year in the safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at $50x10^6$ cells/ml equivalent to $250x10^6$ cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent. In cohort 1a, 50 ml was given per infusion. For cohorts 1b, 2a,2b, 2c and 2d the volume of HepaStem administered will be adapted to the patient's bodyweight.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on the patient's status. The infusion rate shall not exceed 1 ml per min. For cohort 1, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion had to be respected between infusion days. The *Planned* schedule was: in cohort 1a, 250 million cells in 50 ml were administered on each infusion day, leading to a total of 1 billion cells, if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone was expected to be given 15 to 30 min before each HepaStem infusion. The *Actual* schedule is: in cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2a: 3 (up to 6) patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's

	bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2b: 3 (up to 6) patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation). A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort. For cohort 2c: 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2d: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).
Eligibility – Inclusion criteria	<u>Inclusion criteria</u>: 1. Adult aged between 18 and 70 years old. 2. Informed Consent. N.B: In case of hepatic encephalopathy, if the patient is not able to understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement. 3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan).

	4. Patient with Acute Decompensation of cirrhosis 5. Serum total Bilirubin ≥ 6 mg/dL (≥100 umol/L)
Eligibility - Exclusion Criteria	<u>Exclusion criteria</u>: 1. Thombosis of the portal vein. 2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion. 3. Ongoing uncontrolled bleeding. 4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 5. Clinical evidence of Aspergillus infection. 6. Circulatory failure defined by inability to maintain a mean Blood pressure ≥ 70 despite use of vasopressors 7. Mechanical ventilation due to respiratory failure 8. Coagulation disorders defined as: • Fibrinogen < 80 mg/dL • Platelets < 40.000/mm^3 9. Major invasive procedure within 1 week before the infusion (including but not limited to transjugular liver biopsy) 10. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period. 11. MELD score > 35. 12. Previous organ transplantation and/or ongoing immunosuppressive treatments. 13. Postoperative-decompensation following hepatectomy. 14. Renal failure due to chronic kidney disease. 15. Clinically significant left-right cardiac shunt. 16. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions. 17. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. In case of suspicion of HCC, all exam should be done to confirm or not the diagnosis prior enrolment. 18. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.

	19. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 20. Participation to any other interventional study within the last 4 weeks. 21. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisousness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28. The relationship will be assessed based on investigator assessment, and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline. <u>Secondary Endpoints</u>: • Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Mortality ○ Liver transplantation ○ Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score, Child Pugh score. • Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Bilirubin, creatinine, INR and albumin values • Long term safety follow-up ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1 ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of the transplantation, New ACLF episode will be summarized at Month 3 and Year 1
Study Assessment visits	<u>Study visits</u> During the screening and treatment period, patients will be hospitalised. During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

	Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. On HepaStem infusion days, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, TEG (if already performed as part of the clinical routine and up to investigator's judgment), coagulation factors (intrinsic and extrincsic pathway), a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day (even if HepaStem infusions are prematurily stopped). The careful evaluation of these parameters will allow or will not allow the infusion (see section 4.4 – Criteria for treatment discontinuation). On the other days during the hospital stay, patients will be followed-up according to usual practice. A study visit will be performed on Day 4, 8, 12 and 14 $\pm$ 2 days post 1st infusion, including the evaluations listed below. After the treatment period, study visits will be done on days 21 and 28 ($\pm$ 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged. After the active study period, patients will enter the long term follow-up period, including visits at Month 2 ($\pm$ 2 weeks), Month 3 ($\pm$ 2 weeks), Month 6 ($\pm$ 2 weeks), and Year 1 ($\pm$ 1 month). Up to Day 28 visit, all SAEs will be collected. After Day 28 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of the transplantation, malignancies, new AD and/or ACLF episode, AEs assessed by the investigator as possibly related to HepaStem (see Section 7.1.2).

	At the Month 12 study visit, patients will be invited to be included in the Patient long-term follow-up registry.
	
	<u>Study assessments</u>
	
	• All AEs up to Day 28
	• All AESI up to 1 Year.
	• Concomitant medication modifications up to Day 28
	• Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD score (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12,, 14, 21, 28, Months 2, 3. 6 and 12
	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
	  ○ GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
	  ○ Creatinine, Urea or BUN
	  ○ CRP
	  ○ INR, aPTT
	  ○ Serum albumin, sodium, potassium
	• Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
	  ○ INR
	  ○ aPTT
	  ○ fibrinogen
	  ○ D-Dimers
	  ○ TEG (optional, only if measurement can be done locally and up to investigator's judgment)

	• Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion. • Lipase: at screening • Viral serology (HIV, HCV, HEV, HbS antigen) and Aspergilus detection: at screening (if not performed during same admission) • Urine test (Sediment, Creat, Glc, Protein, Albm): at screening • Protein C, Protein S, anti-thrombin III: at screening. In case of deterioration of the coagulation, these measurements will be repeated. • Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2b), 12, 14, 21, 28 (blood testing in central lab) • Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab) • Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab) • Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also at M12.Chest x-ray : at screening (if not performed during same admission) and at M12, • Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected) • ECG: at screening (if not performed during same admission). Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12. Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion. In case of premature withdrawal from study, an end of study visit should be perfomed if possible at the time of study withdrawal. In case of liver transplantation during the course of the study, a sample of the explanted liver will be collected if possible.
Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem is

	administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist. The 3 first patients infused (cohort 1a) received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. 2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage to a new low dose. (see section 1)). The next 3 patients will receive a lower dose of HepaStem and the next 12 (up to 15) patients (new high dose cohort) will receive the higher dose. The total sample size consideration will be a total of 18 (up to 21) patients.
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who received at least one infusion. All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

	All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of the transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 days active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 days active study period or have died or have been lost to follow-up. The Report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Period	**Screening Period**	**Active period**										
	Baseline	**Treatment Period**					**Surveillance Period**		***Long term follow-up***			
Time	**Over 1-7 days prior D1**	Infusion D1	D4 ± 2 days	D8 ± 2 days	D12 ± 2 days	D14 ± 2 days	**D21** ± 2 days	**D28** ± 2 days	**M2** ± 2 weeks	**M3** ± 2 weeks	**M6** ± 2 weeks	**M12** ± 1 month
Informed Consent	X											
Eligibility criteria	X											
Demography & Medical History	X											
Physical exam	X	Xa	X	Xa	X	X	X	X	X	X	X	X
Vital Sign	X	Xb	X	Xb	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD, MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	X%	X	X%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	X%	X	X%	X	X	X	X	X	X	X	X
Lipase & Coagulation 2: C-protein, S-protein, Anti-Thrombin III	X											
Coagulation 1 : INR, aPTT	X	X+	X	X+	X	X	X	X	X	X	X	X
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen	X	X+		X+								
Coagulation 3 : D-Dimers, optional local TEG		X+		X+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	Xa		Xa		X		X				
TEG, TG	X	X*	X	X*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	Xa	X	Xa	X	X	X	X				X
Chest X-Ray	©											X
ECG	©											
Cardiac US Doppler	©	≠										X
Blood culture or other fluid culture	c											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶ cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X										
Cohort 2a & b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2b only								
Cohort 2c & d : Infusion of 1.0.10⁶ cells /kg body weight with a maximum of 100.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2d only								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a: On infusion day: before infusion

b: On infusion day: before, during and after infusion

%: On infusion day: all parameters are measured prior infusion/platelets measurement to be performed prior and post infusion at 4h, 24h, 48h & 72h

C: Only if performed during the same admission

+: On infusion day: prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion

&: On infusion day: prior and 24h after infusion

©: if not already performed during same admission. If already performed, results collected

*: On infusion day: prior and 4h after infusion

AESI: Only Adverse Event of Special Interest to be reported

≠: cardiac US to be performed after infusion

1. BACKGROUND AND RATIONALE

1.1. CIRRHOSIS, ACUTE DECOMPENSATION AND ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Cirrhosis is a progressive chronic liver disease characterized by diffuse fibrosis, severe disruption of the intrahepatic venous flow, portal hypertension and liver failure. The course of cirrhosis is divided into two stages. Compensated cirrhosis defines the period between the onset of cirrhosis and the first major complication. During this period, which is relatively long in most patients (>10 years), symptoms are absent or minor, but liver lesions and portal pressure steadily progress. The term decompensated cirrhosis defines the period following the development of ascites (that is, the accumulation of large amounts of fluid within the peritoneal cavity), variceal haemorrhage and/or hepatic encephalopathy. This period is associated with short-term survival (3–5 years). It is increasingly evident that patients rarely die as a consequence of an end-stage irreversible destruction of the liver. Rather, in most patients, the cause of death is an acute deterioration in their chronic clinical condition promoted by a precipitating event — a syndrome termed acute-on-chronic liver failure (ACLF) (Arroyo et al. 2015).

It is of note that definitions on ACLF may differ worldwide. Given the heterogeneity and the importance of identifying patients four major societies/organisations have provided working definitions (APASL, NACSELD, WGO and EASL-CLIF). The common definition of ACLF is 'a syndrome characterised by acute decompensation of chronic liver disease associated with organ failure(s) and high short-term mortality'. According to the CLIF-ACLF definition developed based on the CANONIC study, ACLF is a recognised syndrome characterised by acute decompensation of cirrhosis associated with the failure of one or more organs and, in the more severe cases, system failure. The organs and systems most likely to fail are the liver, kidney, brain, coagulation, circulation and/or lungs. Patients have a high short term mortality of over 15 % at 28 days (Hernaez R et al, 2017). In the CANONIC study approximately 31% of patients admitted to a hospital for Acute Decompensation (AD) of cirrhosis had ACLF at admission (20%) or developed the syndrome during hospitalisation (11%). The common causes of acute decompensation of liver function included bacterial infections, alcoholic hepatitis, and gastrointestinal hemorrhages, but, in more than 40 % of patients, no precipitating event was identified (Moreau et al. 2013). Among patients with Acute Decompensation (AD), subgroups were identified as being at higher risk of progressing to full blown ACLF and thus at higher mortality risk (Arroyo et al. 2015).
Different grading/scoring systems have been developed in order to better determine prognosis and effectiveness of intervention and care. (Hernaez R et al, 2017).

In daily practice, MELD and Child Pugh scores are still strongly relied on to guide clinical care.

The Model for End-Stage Liver Disease, or MELD, is a scoring system for assessing the severity of chronic liver disease. This score is used by the United Network for Organ Sharing (UNOS) and Eurotransplant for prioritizing allocation of liver transplants. New MELD uses the patient's values for serum bilirubin, serum creatinine, sodium and the international normalized ratio for prothrombin time (INR) to predict survival.

Mortality and MELD score are linearly correlated amongst patients with end-stage liver disease listed for OLT with 3-month mortality estimated to be 4%, 27%, 76%, 83%, and 100% for MELD scores of <10, 10–19, 20–29, 30–39, and 40 or more respectively.

The Child–Pugh score is used in clinical practice to assess the prognosis of chronic liver disease, mainly cirrhosis. It was previously used for prioritizing allocation of liver transplants. The score employs five clinical measures of liver disease: total bilirubin, serum albumin, prothrombin time, ascites and hepatic encephalopathy. Each measure is scored 1–3, with 3 indicating most severe derangement. This leads to three Classes with one year overal survival of 100% for Class A, 81% for class B and 35% for class C. (see 11.6)

ACLF has been defined by the CLIF research consortium into four grades based on retrospectively fitting data on severity linked to mortality score (Moreau et al. 2013) (Table 1-1 and Table 1-2)

- ACLF grade 0 concerns 69.1 % of patients admitted to hospital with acute decompensation. The group is defined as no organ failure, single "non kidney" organ failure (ie, single failure of the liver, coagulation, circulation, or respiration) who had a serum creatinine level < 1.5 mg/dL and no hepatic encephalopathy, or as single cerebral failure with a serum creatinine level < 1.5 mg/dL. These patients have a 28-day and 90-day mortality rate of 4.7% and 14% respectively.
- ACLF grade 1 concerns 15.8 % of patients admitted to hospital with acute decompensation. The group is defined as single kidney failure or single non-kidney organ failure with an organ dysfunction (kidney or brain) and has a 28-day mortality rate of 23 %.
- Patients with ACLF grade 2, defined as two failing organs (10.9 % of patients admitted to hospital with acute decompensation) has an intermediate prognosis (28-day mortality rate of 31%).
- Finally, ACLF grade 3, defined as three or more organ failures (4.4 % of patients admitted to hospital with acute decompensation) has extremely high mortality rates, reaching 75 % after 28 days.

Among patients hospitalised with acute decompensation (AD) (pre ACLF according to the CLIF criteria but ACLF according to other classification systems), an analysis revealed five independent variables including age, serum sodium, white cell count, creatinine and INR as useful for defining a scoring system. The high-risk group (CLIF-C AD score > 60) and intermediate risk group (CLIF-C AD score 46-59) respectively have a 3-month mortality of over 30 % and between 2-30 %. The low risk AD group has a 3-month mortality below 2 % (Arroyo et al. 2015).

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years.The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

Cirrhotic patients with acute decompensation can only receive supportive treatments, such as antibiotics in case of infection, lactulose in case of encephalopathy, terlipressin and albumin in case of hepatorenal syndrome. However, at this moment, there are no treatments available to stop the inflammatory cascade often accompanying the acute decompensation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion,

which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 , renal replacement or use of vasopressors for Hepatorenal syndrome indication
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may cause necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver transplant rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

Conclusion on patient population: Based on this information, Promethera Biosciences proposes that the patient population is defined as cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 µmol/L) and MELD < or = 35.

Patients should have coagulation parameters within the ranges below:

- Fibrinogen ≥ 80 mg/ dL
- Platelets ≥ 40.000/mm3

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidence supports the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properties of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells (similar cells to HHALPC) were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013).

Later, the technology of large-scale cell production was transferred to Promethera Biosciences where clinical batches of HHALPC are produced in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs).

Toxicology *in-vitro or in-vivo* studies aiming to demonstrate the safety, tolerability and tumorigenicity aspect of HepaStem were conducted. *In vivo* studies were performed in rats and mice. They included one study to assess the safety of the intravenous mode of administration. Two studies specifically assessed the risk of tumor formation as this risk has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. An *in vitro* study the pro-coagulant activity of HepaStem was confirmed (Please refer to the IB for more details).

In addition, *invitro* studies show that HepaStem cells express variable immunomodulatory surface markers of interest and have immunomodulatory functional effects: HepaStem inhibits the proliferation of activated T-lymphocytes and blocks the maturation of monocytes (see Section 1.2.6). Furthermore, 6 *in-vivo* studies were conducted with HepaStem evaluating the immunomodulatory properties using the IV route of administration and mainly doses of 12.5 10^6 cells/kg. No safety signal was detected based on these *in vivo* studies.

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg) (4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did

undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. These data laid the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

Based on literature data and Promethera experiences, it can be concluded that liver-derived MSCs, including HepaStem have a pro-coagulant activity. This pro-coagulant activity is also expressed by other MSCs. The pro-coagulant activity might be linked to tissue factor expression, an activator of the coagulation cascade. The procoagulant effect could be modulated by the concomitant administration of bivaluridin during HepaStem infusion in UCD clinical trials in order to prevent, mainly, anticipated thrombotic events. Very high cell doses have been administered intra-portally in the UCD studies in which thrombotic events only occurred at high doses (range: 115 million to 4,1 billion total cells were administered in the portal vein as a split dose in 1 to 10 infusions spread over 1 to 4 consecutive days). Bivaluridin will not be used in the ACLF clinical study as its use has not been validated for late stage cirrhotic patients. Contrary to patients with urea-cycle disorders, coagulation disturbances are common in the late stage chronic cirrhosis population and are linked to liver insufficiency. (see 1.2.5)

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

In a first-in man cohort, conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells (ADHLSC, similar cells to HHALPC) infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated this cell infusion well, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells

repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, spleen, right ankle, and spine, and had disappeared from the lungs. This is in line with the bio-distribution of another type of MSCs administrated in patients (BM-derived MSCs) that demonstrate a similar bio-distribution, with a first pass through the lung; within 24 hours, cells are mainly found in liver, spleen, kidneys and other inflamed areas, by 48 hours, more pronounced presence in the liver is observed. (NDS dossier remestemcel-L, Health Canada).

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For example, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012).

In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

It is clear from consultation of literature publications on cell therapy administration in advanced liver disease including decompensated liver cirrhosis and ACLF that doses of cell therapy protocols tended to be lower as compared to the normal range administered to patients in other immune-modulatory or anti-inflammatory protocols.

The doses and regimens administered to treat patients with chronic liver diseases, range from 0.03 over 0.5 to 1 million MSC cells/kg bodyweight. Different regimens were applied with repeated dosing up to 3 times for the lowest doses (0.03; 0,05 and 0,5 million cells/kg BW repeated 3 times). Most protocols administered the cell infusion intravenously although also other routes of administration were investigated such as intra-splenic, hepatic artery, intrahepatic, intra-lesional route of administration or central venous catheter into the femoral vein. (Berardis et al. 2015)

Based on the literature review, MSC administration is considered to be safe due to the lack of reports of significant adverse effects in the above studies, although a marked heterogeneity was observed among studies with regard to injection dose, frequency of injection, cell source, delivery route and study design. Most of these early studies reported improvements in liver function, ascites and encephalopathy.

In the first cohort of 3 patients in the HEP001 study the lowest dose (12.5×10^6 cells/kg) of the range of doses administered safely in previous studies of HepaStem in urea cycle disorder (UCD) and Crigler Najar pediatric patients was used to determine the dose in the HEP101 protocol. The (low) dose proposed (250

million cells/ infusion; ie. 3.5 10^6 cells/kg BW/infusion) was a reduction of 4x of the lowest dose tested previously (in the HEP001 protocol) and it was thought that it could be safely administered in cirrhotic patients. Additionally, the number of cells administered per infusion would be limited, similar to MSC doses given in immune mediated inflammatory diseases. In retrospect, it was clear that adaptation to the dose level similar to other MSCs given in immune-mediated inflammatory diseases was inadequate and did not take the specific case of severely ill chronic cirrhotic patients with acute decompensation into account.

Therefore, it seems that using a careful approach starting with doses commonly used in reported studies of decompensated cirrhosis and ACLF patients and published as being safe, appears to be an acceptable approach. Also, dose escalation to a maximum of 1.0 million cells/kg BW per infusion should be feasible based on a repetitive dosing schedule. In case of repetitive dosing, the doses will be given weekly, which allows time in case of fibrinolysis for the parameters to be corrected and return to normal.

Pre-clinical immunomodulatory data of liver-derived progenitor cells

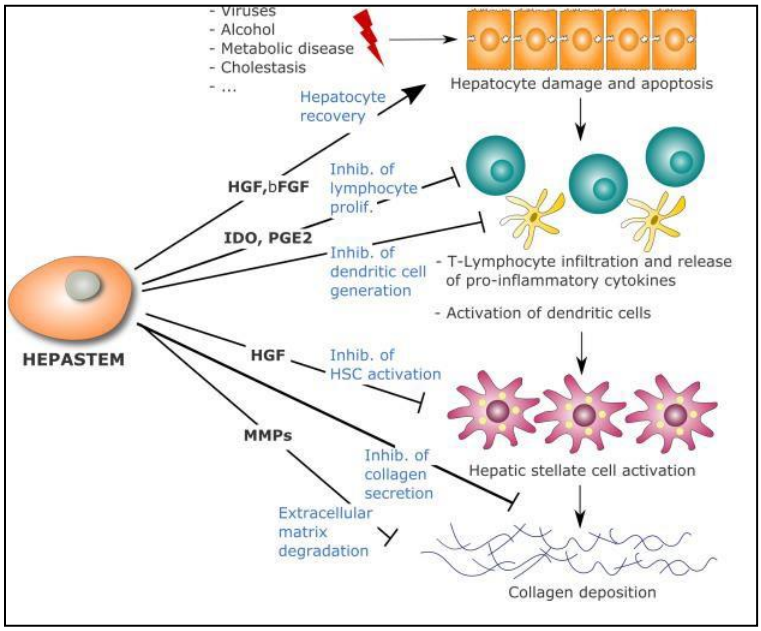
The first transcriptomics and secretomics tests performed on liver-derived progenitor cells (similar cells to HHALPC) grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (similar cells to HHALPC) (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature

dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models presents important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.6. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing to the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic

hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.7. Expected Benefits of HepaStem

Proposed mechanisms of action: after intravenous administration of HepaStem, cells are expected to circulate into the blood network where they can exert a systemic immunomodulatory action. At the same time, they have a main homing into the liver where they can thus also exert some important local immunomodulatory effects. They are expected to play their immunomodulatory roles through direct cell-to-cell interaction and through paracrine effects via the various cytokines, chemokines, MMPs and growth factors they may secrete. HepaStem could affect monocytes and DC recruitment, differentiation, maturation and function through cell contacts or paracrine signalling. HepaStem could also alter the proliferation and activation of T-lymphocytes that are another dysregulated cell type of the immune system in ACLF. In addition to modulate the behaviour of immune cells, HepaStem could modulate the proliferation and activation of hepatocytes and hepatic stellate cells and thus their secretory profiles, helping in this way the liver function recovery. The current *in vitro* and *in vivo* data, based on the scientific literature, and sponsor *in vitro* results, support all these potential immunomodulatory effects of HepaStem in ACLF patients.

Proposed clinical significant benefit: by these combined effects, HepaStem could play a favourable role in restoring an immunological balance in ACLF patients or patients at risk of ACLF, improving organ failure scores, improving clinical status, possibly leading to a resolution of this acute event and demonstrating improvement of transplantation free survival.

Considering the unmet medical need: i. the emergency to treat cirrhotic patients with Acute Decompensation (pre-ACLF or ACLF) due to the high mortality rate; ii. the shortage of healthy donors and the need of livers in the context of liver transplantation; iii. Concerns raised recently regarding artificial liver support; and iv. the mechanism of action of HepaStem, we can say that all these factors are in favour of a promising favourable benefit/risk balance for HepaStem. The exact profile of which patients will benefit most is under investigation, and also subject of this safety study.

1.2.8. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts with 18 (up to 21) hospitalized patients in total. The study will include patients with an acute decompensation of cirrhosis and with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 35, excluding patients with circulatory, respiratory failure or severe coagulations disorders. It is planned to have a first group of 6 patients (cohort 1) being administered with the low dose.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days.

On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 patients experienced an episode of severe bleeding. Therefore, it has been decided to reduce the dose in the low dose cohort to $0.25.10^6$ cells/kg bodyweight with a maximum of 25.10^6 cells in a single infusion. A reduction of minimum 10 times the dose previously used.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

Once this has been proven safe, a second group of up to 6 patients (cohort 2a) will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: Up to 6 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort.

For cohort 2c: 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2d: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).

HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period.

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 12 patients, appears to be an acceptable approach in patients with ACLF or with acute decompensation at risk of developing ACLF for whom no specific therapeutic or curative treatment exist. (See section 1.1)

HepaStem administration will be started rapidly after hospitalisation and will be completed within 1 day (cohort 1b, 2a, 2c) or within 1 week (cohort 2b, 2d). This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of patients with or at risk of developing ACLF. As ACLF and/or Acute Decompensation of cirrhosis is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up up to 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining the design of future efficacy clinical studies.

Study population

The patient population is defined by cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 35 (see section 1.1).

Patients should have coagulation parameters within the ranges below:

- Fibrinogen ≥ 80 mg/ dL
- Platelets ≥ 40.000/mm3

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort 1a- corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 14 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100 mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 28 million cells/kg (assuming a patient

weight of 70 kg). The first selected dose (250 million cells per infusion) is close to MSC doses given in other trials for immune-mediated inflammatory diseases (Section 1.2.5), therefore, it was expected to show a similar safety and efficacy profile. It corresponded also to the high dose of liver-dirived progenitor cells (similar cells to HHALPC) administered via IV to the heamophila patient (see 1.2.4).

Due to the severe bleeding that occurred in 2 of the 3 patients that received 250 10^6 cells (50 mL of HepaStem) per infusion, the next selected dose (low dose cohort 1b) will be reduced to 0.25.10^6cells/kg bodyweight (with a maximum of 25.10^6 cells per infusion) administered in a single infusion (at least a 10x reduction of the dose administered in cohort 1a).

The second selected dose represents a two-fold increase from the dose in cohort 1b (0.5.10^6cells/kg bodyweight (with a maximum of 50.10^6 cells per infusion) still in the range of doses reported for MSCs and more in the range of doses administered in the specific case of severely ill chronic liver disease patients with ACLF and acute decompensation of cirrhosis. (see1.2.5)). (for additional information, please refer to the rationale for changes).

The third and fourth selected dose represents a two-fold increase from the cohort 2a and 2b. These dose still in the range of doses reported for MSCs and more in the range of doses administered in the specific case of severely ill chronic liver disease patients with ACLF and acute decompensation of cirrhosis. (see1.2.5)). (for additional information, please refer to the rationale for changes dated 28June2018).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 IU/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damage nor any respiratory symptoms, before homing mainly to the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF, it also can allow repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs also have a procoagulant activity comparable to liver-derived progenitor cells (similar cells to HHALPC) (Stephenne et al. 2012),

nevertheless literature reports show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015).

However, liver failure results in a state of "rebalanced hemostasis" marked by a decrease in both pro-coagulation and anticoagulation factors. Patients with severe liver disease are not auto-anticoagulated. In essence, patients with severe liver disease, acute and/or chronic, have a tenuous rebalanced hemostasis that is easily perturbed by various disease states and concomitant medications and invasive procedures. Bleeding events including severe forms are common in these end-stage liver disease patients. The events of epistaxis and bleeding from puncture sites that occurred in 2 patients in cohort 1a (in retrospect a high dose in late stage cirrhotic patients), have been recognised in the literature as case reports. It was also stated that epistaxis as an overlooked cause of massive haematemesis in cirrhosis should be added to the list of upper GI bleeding (Johal et al 2003). Hence, cirrhotic patients including ACLF patients are at increased risk of bleeding or thrombosis. Therefore, dose reduction from normal ranges applied in other immune-modulatory diseases, modification of inclusion criteria and increased surveillance of liver and coagualation parameters is indicated.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of different regimens of HepaStem in cirrhotic patients presenting with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of different dose regimens of HepaStem given in subsequent cohorts with 18 (up to 21) hospitalized patients in total.

5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next patients enrolled will complete each dose cohort in a step wise approach and will receive a lower dose following SAEs observed in patient 2 and patient 3 in the cohort 1a with high dose of cells).

Twelve (up to fifteen) other patients will be enrolled in cohort 2.

3 (up to 6) patients of the cohort 2 (cohort 2a) will receive twice the dose compared to the cohort 1b.

3 (up to 6) patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort.

The 3 patients of the cohort 2c and 2d will receive up to 2 times the dose given in to the cohort 2b.

The study will recruit patients who are hospitalized for Acute Decompensation of cirrhosis and/or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

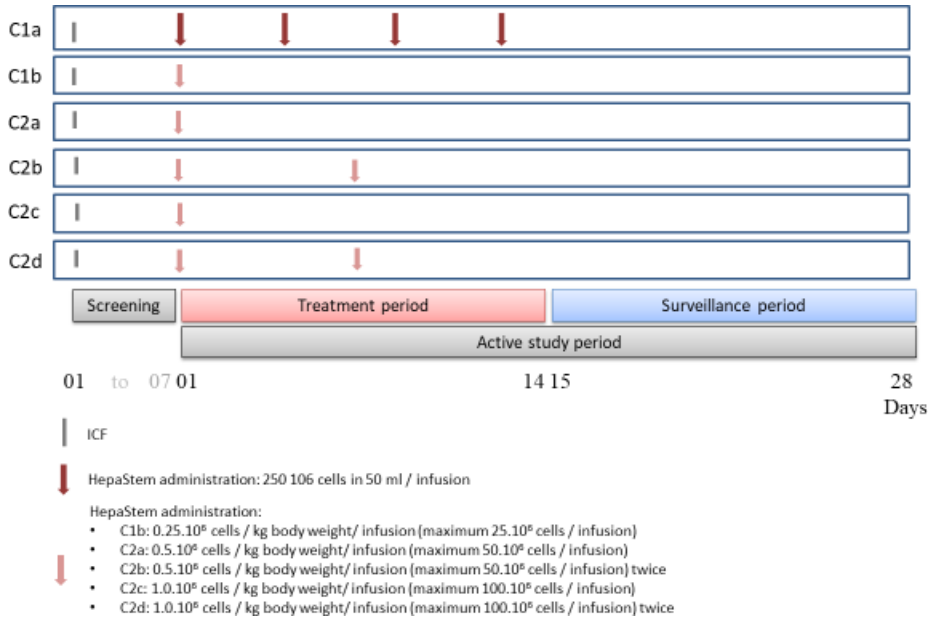
Screening period: Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days). The duration of the screening period plus the active period will last up to 35 days (± 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



Planned schedule:

For cohort 1a, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion must be respected between infusion days.

In cohort 1a, 250 million cells in 50 ml were administrated on each infusion day, leading to a total of 1 billion cells if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before each HepaStem infusion.

Actual schedule:

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

Planned schedule:

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2a: 3 (up to 6) patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 (up to 6) patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).

A minimum of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of this protocol before to start inclusion in the next dose cohort.

For cohort 2c: 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2d: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).

Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of members, all external and independent to Promethera Biosciences. (See Section 9.13):

In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

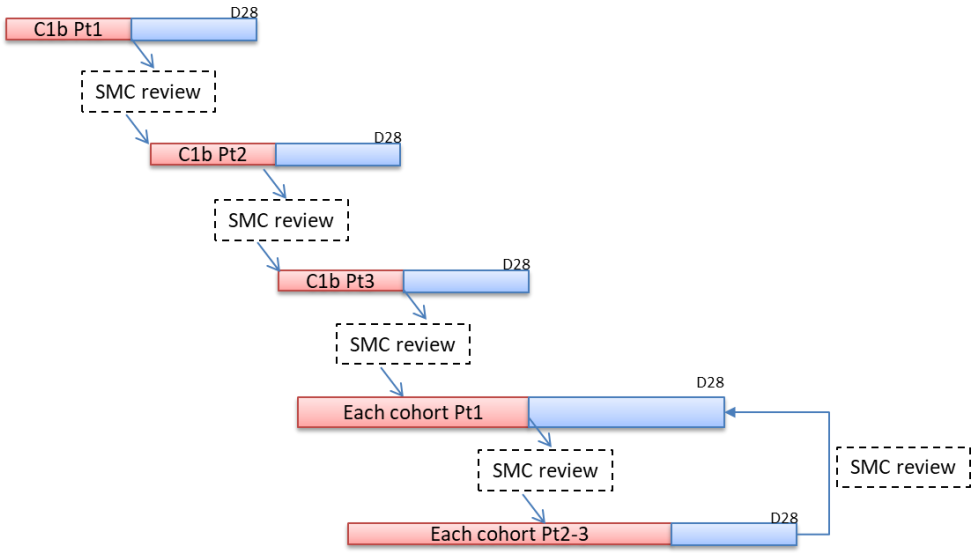
As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a, 2b, 2c and 2d), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- For the first cohort (1b), when the second evaluable patient has received HepaStem infusion (complete scheme or premature stop), the patient data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patients' parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a single administration of $0.5.10^6$ cells/kg body weight).

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring committee



The study assessments are described in Section 6.

Furthermore, the 3 first patients of the cohort 2a,2b, 2c and 2d will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete scheme or premature stop). The sequential approach will be under the control of Promethera (based on review of eligibility criteria by the medical monitor and HepaStem delivery). In case of safety signal, the SMC will be involved in the AEs review and evaluation, and the SMC will advise on further inclusion.

These measures (SMC meetings and sequential treatment for the 3 first patients in each cohort) will allow respecting the progress of dose levels with limited risk for the patients.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the safety follow-up period.

After completion of this study, patients will be invited to be followed-up in the Patient Long-term follow-up registry for 5 years.

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
    - Mortality
    - Liver transplantation
    - Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
    - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
    - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of the transplantation
- New ACLF episode will be sumamrized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in up to 25 centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (35 days ($\pm$ 2 days) in the screening plus active treatment period), and therafter for an additional period of 1 year in the safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. The hospitalisation unit will be adapted according to the medical status of the patient and the organization of study center hospital. Patients with a low CLIF-OF score will be more likely included in the hepatology department (standard or intermediate care unit), while the patient with high CLIF-OF score will more likely be included in the Intensive Care Unit.

Patients will remain hospitalised at least during the treatment period. During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Cirrhotic patients with Acute Decompensation at risk of developing ACLF at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last maximum 7 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 years old.
2. Informed Consent.
   N.B: In case of hepatic encephalopathy, if the patient is not able to understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.
3. Cirrhosis as diagnosed by
   - liver histology or
   - clinical and imaging examination (may include fibroscan).
4. Patient with Acute Decompensation of cirrhosis
5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Thrombosis of the portal vein.
2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.

   Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.
3. Ongoing uncontrolled bleeding.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
5. Clinical evidence of Aspergilus infection.
6. Circulatory failure defined by inability to maintain a mean Blood pressure ≥ 70 despite use of vasopressors.
7. Mechanical ventilation due to respiratory failure.
8. Coagulation disorders defined as :
   - Fibrinogen < 80 mg/dL
   - Platelets < 40.000/mm^3
9. Major invasive procedure within the week before the infusion (including but not limited to tranjugular liver biopsy)
10. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.
11. MELD score > 35.
12. Previous organ transplantation and/or ongoing immunosuppressive treatments.
13. Postoperative-decompensation following hepatectomy.
14. Renal failure due to chronic kidney disease.
15. Clinically significant left-right cardiac shunt.
16. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
17. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. In case of suspicion of HCC, all exam should be done to confirm or not the diagnosis prior enrolment.
18. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
19. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
20. Participation to any other interventional study within the last 4 weeks.
21. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration for a patient are the following:

- **Transitory discontinuation:** Coagulation disorders considered as significant (Fibrinogen < 80 mg/dL, or Platelets < 40.000/mm^3) by the PI prior to each infusion should preclude the administration of Hepastem.
- Thrombosis of the portal vein should preclude the administration of Hepastem.
- Tranfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.

Infusions may be restarted after recovery. If planned infusions are not performed within treatment period ($\pm$ 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock, severe worsening of hepatic function.

The reason of study treatment discontinuation will be documented, and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAWAL CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).
- The exact dosage (volume) of Hepastem infused to the patient will be calculated based on the weight of the patient on the day of infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells/infusion (5 mL) for cohort 1b, $0.5.10^6$ or $1.0.10^6$ cells per kg bodyweight with a maximum of 50.10^6 or 100. 10^6cells/infusion (10 mL) for cohort 2.)
- As the exact volume to infused can be low (depending on the patient's weight), it is recommended to flush after the infusion physiological solution (NaCl 0,9%) to ensure that all the product is infused.

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and the syringe will

be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given.

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells per infusion). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapated accordingly. A single infusion is expected to last minimum 5 min (for 5 mL of reconstituted Hepastem).

For cohort 2a and 2b: 3 (up to 6) patients will receive HepaStem in a single infusion (cohort 2a) or in 2 repeated infusions one week apart (cohort 2b). The dosage of Hepastem per infusion will be $0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion).

For cohort 2c and 2d: 3 patients will receive HepaStem in a single infusion (cohort 2c) or in 2 repeated infusions one week apart (cohort 2d). The dosage of Hepastem per infusion will be $1.0.10^6$ cells per kg body weight with a maximum of 100.10^6 cells per infusion).

Each infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single infusion is expected to last maximum 20 min (for 20 mL of reconstituted Hepastem).

The full procedure describing how to adapat the volume of HepaStem to be administrated to the patient's body weight is in the HepaStem Manual.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate, ICUs or standard unitsdepending on the medical status of the patient and the organisation of study center hospital. Regardless of the unit of hospitalization, patients will remain hospitalised at least during the treatment period, with a close monitoring of each patient. During HepaStem infusion, a continuous monitoring of the vital signs of the patient is required.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a

specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow and to exclude thrombosis of the portal vein (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension has to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation : the syringe has to be inversed 10 times every 3 minutes.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics, risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), and risks observed with the infusion of HepaStem in the cohort 1a, main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis or to consumption of coagulation factors and subsequent bleeding; respiratory disorder as cells first transit to the lungs; hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the risks mentioned below, there might be other, at this time, unknown risks.

5.4.1. Risk and Benefit assessment

ACLF patients or patients at high risk to develop ALCF are at high mortality risk and there is currently no specific treatment for these patients. Orthotopic liver transplantation is often not a possible option for these patients. By its potential combined effects, HepaStem could play a favourable role in restoring an immunological balance in pre-ACLF / ACLF patients, leading to a resolution of this acute event and showing

improvement of organ function and transplantation free survival. The main identified risks linked to HepaStem are activation of the coagulation cascade and may lead to thrombosis (observed in UCD patients) or bleeding (observed in ACLF patients). The safety measures described below (see section 5.5) are recommended to minimize the risks of the administration of HepaStem in ACLF or pre-ACLF patients at high risk of short term mortality.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the

Before each infusion, the investigator will have to make sure the patient has the minimum criteria to receive HepaStem (see 4.4 - Criteria for study treatment discontinuation).

Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lung disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect.

Number of cells administered for the cohort 1a, 2a,2b, 2c and 2d per infusion will be maximum 25.10^6 cells (cohort 1a) 50.10^6 cells (cohorts 2a and 2b) or 100.10^6 cells (cohorts 2c and 2d)and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min. The lower dose regimen will be applied before the higher one. These doses are in the very low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication and re-adjusted to the range of MSC doses given in decompensated cirrhotic and ACLF patients (See Section 1).

Furthermore, **the coagulation parameters will be closely monitored** prior and after the infusion process at 4h, 8h, 12h, 24h, 48h and 72h post infusion. (Including INR, aPTT, fibrinogen, D-Dimers, coagulation factors (pre and 24h post infusion), and TEG (optional, only if measurement can be done locally and up to investigator's judgment)

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion, and patient with risk of bleeding (defined by recent major invasive procedure, non controlled gastrointestinal hemorrhage and/or coagulation disorders) will be excluded from the study.

In case major changes in the coagulation parameters and/or clinically significant bleedings suggestive of important coagulation factors consumption occur, according to the investigator's judgement, it could be envisioned to administer coagulation factors in the form of fresh frozen plasma (FFP), fibrinogen concentrate (ie RiaSTAP), and/or antifibrinolytics (ie tranexamic acid). (cfr. both study patients in cohort 1a responded well to treatment with FFP and/or addition of coagulation factors).

In case of clinical signs or suspicion of pulmonary embolism, the investigator should perform exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigraphy.

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions. Any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement.

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours and/or additionnal exams if needed (abdominal CT scan or MRI) to exclude thrombosis of the portal vein. **Hepastem will be administred only if portal vein patency is demonstrated (excluding thrombosis of the portal vein).**

Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.2. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.3. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.4. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.5. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Safety follow-up Period of 1 year.

Thereafter, patients will be invited to be followed-up in the Patient long-term safety follow-up Registry for 5 additionnal years

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before first HepaStem infusion of the day.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and treatment period, patients will be hospitalised in intermediate or ICUs or standard units, depending of the severity of the patient disease.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1

On HepaStem infusion days, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, coagulation factors, TEG (if already performed as part of the clinical routine and up to investigator's judgment) a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

The careful evaluation of these parameters will allow or not the infusion (see section 4.4 – Criteria for treatment discontinuation).

During the infusion, the patient will be continuously monitored for early detection of any potential AEs.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

A study visit will be performed on Day 14 $\pm$ 2 days, including the evaluations listed below.

In case of any suspicion of AE, the investigator will perform the exams she/he evaluates as appropriate. In particular, any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement. In case of clinical signs or suspicion of pulmonary embolism, the investigator should perfom exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigrapy.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to the 28 days visit, all SAEs will be collected. After the 28 days visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of the transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be followed-up in the Patient long-term safety follow-up Registry for 5 additional years.

6.2.1. Study assessments

- All AEs up to Day 28
- All AESI up to 1 Year.
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation),MELD score and Child Pugh will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
    - GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
    - INR
    - aPTT

- fibrinogen
- D-Dimers
- TEG (optional, only if measurement can be done locally and up to investigator's judgment)

- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion.
- Lipase: at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening. Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2), 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also on M12
- Chest x-ray at screening (if not performed during same admission) and at M12,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)
- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

<u>Thromboelastogram and thromboglobulin generation tests</u>

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min twice). The plasma will be colleted and put in cryotubes (min. 2 tubes containing each min. 500 µL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Mounier, 53
1200 Woluwe-Saint Lambert BELGIUM

Anti-HLA antibodies

Serum will be collected and send within 48 hours (ambient temperature) to :

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Mounier 53, 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHARTS

Table 6-1 Study Flowchart

Period	**Screening Period**	**Active period**										
	Baseline	**Treatment Period**					**Surveillance Period**		***Long term follow-up***			
Time	**Over 1-7 days prior D1**	Infusion D1	D4 ± 2 days	D8 ± 2 days	D12 ± 2 days	D14 ± 2 days	**D21** ± 2 days	**D28** ± 2 days	**M2** ± 2 weeks	**M3** ± 2 weeks	**M6** ± 2 weeks	**M12** ± 1 month
Informed Consent	X											
Eligibility criteria	X											
Demography & Medical History	X											
Physical exam	X	Xa	X	Xa	X	X	X	X	X	X	X	X
Vital Sign	X	Xb	X	Xb	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD, MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	X%	X	X%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	X%	X	X%	X	X	X	X	X	X	X	X
Lipase & Coagulation 2: C-protein, S-protein, Anti-Thrombin III	X											
Coagulation 1 : INR, aPTT	X	X+	X	X+	X	X	X	X	X	X	X	X
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen	X	X+		X+								
Coagulation 3 : D-Dimers, optional local TEG		X+		X+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	Xa		Xa		X		X				
TEG, TG	X	X*	X	X*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	Xa	X	Xa	X	X	X	X				X
Chest X-Ray	©											X
ECG	©											
Cardiac US Doppler	©	≠										X
Blood culture or other fluid culture	c											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶ cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X										
Cohort 2a & b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2b only								
Cohort 2c & d : Infusion of 1.0.10⁶ cells /kg body weight with a maximum of 100.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2d only								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a: On infusion day: before infusion	b: On infusion day: before, during and after infusion
%: On infusion day: all parameters are measured prior infusion/platelets measurement to be performed prior and post infusion at 4h, 24h, 48h & 72h	C: Only if performed during the same admission
+: On infusion day: prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion	&: On infusion day: prior and 24h after infusion
©: if not already performed during same admission. If already performed, results collected	*: On infusion day: prior and 4h after infusion
AESI: Only Adverse Event of Special Interest to be reported	≠: cardiac US to be performed after infusion

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Serious Adverse Event of Special Interest are defined as:

- AEs with fatal outcome

- Transplantation and outcome of the transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

All SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possible, probable or definite.

7.1.5. Serious Adverse Drug Reactions (SADR)

A SADR is any ADR that is serious as per the above criterias.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

<u>Possibly</u>

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probably

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definitly

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

Moderate, when interfering with ADL;

Severe, when impeding ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Serious Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients

enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SADR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts each with 18 (up to 21) hospitalized patients in total.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

The 3 first patients infused (cohort 1a) : 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage (see section 1).

The next 3 patients cohort will receive a lower dose of HepaStem and the next 12 (up to 15) patients cohorts (high dose cohort) will receive the higher dose.

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Since it is a safety study, any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

The total sample size consideration will be a total of 18 (up to 21) patients.

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied.

Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of thetransplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 days active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The third analysis will be performed after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after treated patients of cohorts 1 and 2 will have completed the 28 days active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], if the patient is not able to understand the study based on the investigator's judgment, the informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant

protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences

must “verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection”.

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator’s Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient’s further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent, or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of members with expertise in liver disease or other relevant medical fields, all external and independent to Promethera. The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.

2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.

As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a, 2b, 2c and 2d), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- For the first cohort (1b), when the second evaluable patient has received HepaStem infusion (complete scheme or premature stop), the patient data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme of premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

More specifically, based on the patient's parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a unique administration of $0.5.10^6$ cells/kg body weight).

5. The SMC will review severe coagulation events assessed as related to HepaStem administration by the investigator.
6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- Expert(s) in hepatology
- Expert(s) in Homeostasis
- One or more biostatisticians
- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists may be invited to participate as non-voting members at any time and Promethera Biosciences members may provide additional information if additional expertise is desired, but are not members of the SMC.

The frequency of SMC meetings will depend on several factors including the rate of enrollment, completion of patients in the dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC and to Regulatory Authority if requested.

10. REFERENCE LIST

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL, rena replacement or use o vasopressors for Hepatorena syndrome indication
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	 >300 >357	 ≤300 - > 200 >214- ≤357	 ≤200 ≤214

Arroyo et al. 2015

11.2. CLIF ACLF GRADE

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

11.3. CLIF-C ACLF SCORE

CLIF-C ACLF = $10 \times [(0{,}33 \times \text{CLIF OF} + 0.04 \times \text{Age} + 0{,}63 \times \text{Ln}(\text{WBC}\{10^9 \text{ cells/L}\}) - 2]$

11.4. CLIF CONSORTIUM ACUTE DECOMPENSATION SCORE (CLIF-C AD)

CLIF-C AD = 10 × [(0,03 × Age {years} + 0,66 × Ln(Creatinine{mg/dL} + 1.71 × Ln(INR) + 0,88 × Ln(WBC{10^9 cells/L}) – 0,05 × Sodium {mmol/L} + 8]

Jalan et al. 2015

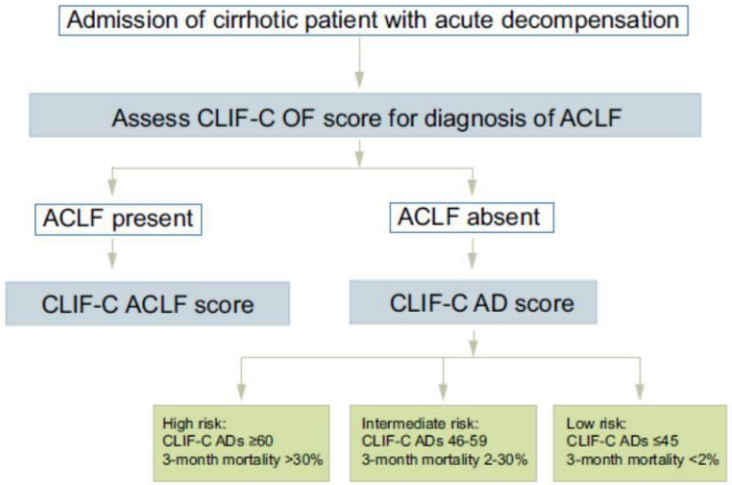

Fig. 4. Algorithm for the sequential use of the EASL-CLIF Consortium predictive scores in patients with cirrhosis admitted to hospital with acute decompensation.

11.5. MELD SCORE

MELD score is calculated using serum bilirubin, serum creatinine, and International Normalized Ratio (INR) and is given by the formula :

MELD(i) = (0.957 * ln(Serum Cr) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10 (if hemodialysis, value for Creatinine is automatically set to 4.0)

MELD Score (2016) = MELD(i) + 1.32*(137-Na) – [0.033*MELD(i)*(137-Na)]

Note: Sodium has a range of 125-137 mEq/L

11.6. THE SCORE CAN BE CALCULATED USING ONLINE WEBSITE HTTPS://WWW.MDCALC.COM/MELD-SCORE-MODEL-END-STAGE-LIVER-DISEASE-12-OLDERCHILD PUGH SCORE

Measure	1 point	2 points	3 points
Total bilirubin, µmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8
Prothrombin time prolongation (s)	<4.0	4.0–6.0	> 6.0

Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	Class	One year survival	Two year survival
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

Singal AK, Kamath PS. Model for End-stage Liver Disease. J Clin Exp Hepatol. 2013 Mar;3(1):50-60. Review.

11.7. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behavior*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 26 June 2018

Version Number: 5.1

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____ _____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 26 June 2018

Version Number: 5.1

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competente Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

Silver Ocean Ventures SAS, CEO, represented by John Tchelingerian Promethera Biosciences	Date
Etienne Sokal, Chief Scientific & Medical Officer Promethera Biosciences	Date

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 6.0 _ 14 December 2018

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
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MONITORING & STUDY COORDINATION	Virginie Barthel, T.+32.471.784.097 Nele Vanparys, T.+34.665.245.704
SAFETY & PHARMACOVIGILANCE	pv@promethera.com **T.*+32.10.39.43.43***

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LIST OF ABBREVIATIONS

AD	Acute Decompensation
ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SADR	Serious Adverse Drug Reaction
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 6.0 – 14 Dec 2018
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Approximately twenty-one (21) evaluable Patients
Number of Centers	Up to 25 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts with approximately 21 hospitalized patients in total 5 patients were screened in the cohort 1a, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next patients enrolled will complete each dose cohort in a step wise approach and will receive a lower dose following SAEs observed in patient 2 and patient 3 in the cohort 1a (with high dose of cells).

Approximately three patients will be enrolled in cohort 1b.

Approximately fifteen patients will be enrolled in cohort 2 (cohorts 2a + 2b + 2c + 2d).

Approximately 3 patients of cohort 2a will receive twice the dose compared to cohort 1b.

Approximately 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

Additionally, approximately 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort.

The patients of the cohort 2c and 2d (approximately 3 patients for each subcohort) will receive up to 2 times the dose given in to the cohort 2b

The statistical analysis will take into consideration the different doses applied.

<u>Study periods</u>

The study will recruit cirrhotic patients who are hospitalized for ACLF or Acute Decompensation at risk of developing ACLF

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Patients with Acute Decompensation of cirrhosis at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining signed informed consent, the screening period may last maximum 7 days, time to complete the screening process and to deliver

	HepaStem to the center. This period will include confirmation of eligibility criteria. The study is divided in the following periods: screening period, active study period divided in treatment period and evaluation period, and long-term safety follow-up. Screening period: Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. Active study period: The active study period will last 28 days (± 2 days). The duration of the screening period plus the active period will last up to 35 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Various dose regimens of HepaStem will be given, which differ in the amount of cells per infusion and/or in the number of infusions. The 3 patients in the cohort 1a received the dose regimen of 250.10^6 cells per infusion – this represents approximately 2.9 to $3.4.10^6$ cells/kg bodyweight in the first cohort. (cohort 1a) Approximately three patients in cohort 1 (cohort 1b) will receive a lower dose (minimum ten times lower) in a single infusion ($0.25.10^6$ cells /kg bodyweight with a maximum of 25.10^6 cells per infusion). Approximately 3 patients in cohort 2 (cohort 2a will receive twice the dose of the patients in cohort 1b ($0.5.10^6$ cells/kg bodyweight with a maximum of 50.10^6 cells per infusion). Approximately 3 patients in cohort 2 (cohort 2b) will receive up to 2 doses of $0.5.10^6$ cells/ kg bodyweight 1 week apart ($0.5.10^6$ cells/kg bodyweight per infusion with a maximum of 50.10^6 cells per infusion). Additionally, approximately 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort.

	Approximately 3 patients in cohort 2 (cohort 2c) will receive twice the dose of the patients in cohort 2a (1.10^6 cells/kg bodyweight with a maximum of 100.10^6 cells per infusion). Approximately 3 patients in cohort 2 (cohort 2d) will receive up to 2 doses of 1.10^6 cells/ kg bodyweight 1 week apart (1.10^6 cells/kg bodyweight per infusion with a maximum of 100.10^6 cells per infusion) Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of members, all external and independent to Promethera Biosciences. As a minimum, the safety data will be reviewed by the SMC at the following timepoints : - For each cohort (1b, 2a, 2b, 2c and 2d), when the first evaluable patient has received the HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients. - When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advice on the enrolment of patients in the next cohort. If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient. More specifically, based on the patients' parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg bodyweight) instead of a single administration of $0.5.10^6$ cells/kg bodyweight). Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion (D1) in the safety follow-up period. After completion of this study, patients will be invited to be followed-up in the Patient long term safety follow up Registry for 5 additionnal years.
Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (35 days ($\pm$ 2 days) in the screening plus active treatment period), and thereafter for an additional period up to 1 year post first HepaStem infusion (D1) in the safety follow-up.

Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at $50x10^6$ cells/ml equivalent to $250x10^6$ cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent. In cohort 1a, 50 ml was given per infusion. For cohorts 1b, 2a, 2b, 2c and 2d, the volume of HepaStem administered will be adapted to the patient's bodyweight.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on the patient's status. The infusion rate shall not exceed 1 ml per min. For cohort 1, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion had to be respected between infusion days. The *Planned* schedule was: in cohort 1a, 250 million cells in 50 ml were administered on each infusion day, leading to a total of 1 billion cells, if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone was expected to be given 15 to 30 min before each HepaStem infusion. The *Actual* schedule is: in cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. For cohort 1b: Approximately 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2a: Approximately 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or

	equivalent (see section 5.6 'Concomitant medication') will be given 15 to 30 min before HepaStem infusion. For cohort 2b: Approximately 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation). Additionally, approximately 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort. For cohort 2c: Approximately 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. For cohort 2d: Approximately 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).
Eligibility - Inclusion Criteria	<u>Inclusion criteria</u>: 1. Adult aged between 18 and 70 years old. 2. Signed Informed Consent. <u>N.B:</u> In case of hepatic encephalopathy, if the patient is not able to understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local

	regulation, and by the patient, if possible, after encephalopathy improvement. 3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan). 4. Patient with Acute Decompensation of cirrhosis 5. Serum total Bilirubin ≥ 6 mg/dL (≥100 umol/L)
Eligibility - Exclusion Criteria	<u>Exclusion criteria</u>: 1. Thrombosis of the portal vein. 2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion. 3. Ongoing uncontrolled bleeding. 4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotictherapy for more than 48h. 5. Clinical evidence of Aspergillus infection. 6. Circulatory failure defined by inability to maintain a mean Blood pressure ≥ 70 despite use of vasopressors 7. Mechanical ventilation due to respiratory failure 8. Coagulation disorders defined as : • Fibrinogen < 80 mg/dL • Platelets < 40.000/mm^3 9. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator. 10. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period. 11. MELD score > 35. 12. Previous organ transplantation and/or ongoing immunosuppressive treatments. 13. Postoperative-decompensation following hepatectomy. 14. Renal failure due to chronic kidney disease.

	15. Clinically significant left-right cardiac shunt. 16. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions. 17. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. In case of suspicion of HCC, all exam should be done to confirm or not the diagnosis prior enrolment. 18. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 19. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 20. Participation to any other interventional study within the last 4 weeks. 21. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisousness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28. The relationship will be assessed based on investigator assessment, and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline. <u>Secondary Endpoints</u>: • Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Mortality ○ Liver transplantation ○ Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score, Child Pugh score. • Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Bilirubin, creatinine, INR and albumin values • Long term safety follow-up

	○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1 ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation New ACLF episode will be summarized at Month 3 and Year 1
Study Assessment visits	<u>Study visits</u> During the screening and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. On HepaStem infusion days, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, TEG (if already performed as part of the clinical routine and up to investigator's judgment), coagulation factors (intrinsic and extrincsic pathway), a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day (even if HepaStem infusions are prematurily stopped). The careful evaluation of these parameters will allow or will not allow the infusion (see section 4.4 – Criteria for treatment discontinuation). On the other days during the hospital stay, patients will be followed-up according to usual practice. A study visit will be performed on Day 4, 8, 12 and 14 $\pm$ 2 days post 1st infusion, including the evaluations listed below. After the treatment period, study visits will be done on days 21 and 28 ($\pm$2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

	After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).
	
	Up to Day 28 visit, all SAEs will be collected. After Day 28 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new AD and/or ACLF episode, AEs assessed by the investigator as possibly related to HepaStem (see Section 7.1.2).
	
	At Month 12 study visit, patients will be invited to be included in the Patient Long term follow up registry.
	
	<u>Study assessments</u>
	
	• All AEs up to Day 28
	• All AESI up to 1 Year.
	• Concomitant medication modifications up to Day 28
	• Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation):
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD score (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3. 6 and 12
	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
	  ○ GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
	  ○ Creatinine, Urea or BUN
	  ○ CRP
	  ○ INR, aPTT
	  ○ Serum albumin, sodium, potassium
	• Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day

	8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment. ○ INR ○ aPTT ○ fibrinogen ○ D-Dimers ○ TEG (optional, only if measurement can be done locally and up to investigator's judgment) • Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion. • Lipase: at screening • Fibrinogen at screening • Viral serology (HIV, HCV, HEV, HbS antigen) and Aspergilus detection: at screening (if not performed during same admission) • Urine test (Sediment, Creat, Glc, Protein, Albm): at screening • Protein C, Protein S, anti-thrombin III: at screening. In case of deterioration of the coagulation, these measurements will be repeated. • Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2b), 12, 14, 21, 28 (blood testing in central lab) • Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab) • Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab) • Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also at M12. • Chest x-ray : at screening (if not performed during same admission) and at M12, • Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected) • ECG: at screening (if not performed during same admission). • Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12. A SMC will review safety data and advise on study conduct.

	Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion. In case of premature withdrawal from study, an end of study visit should be perfomed if possible at the time of study withdrawal. In case of liver transplantation during the course of the study, a sample of the explanted liver will be collected if possible.
Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).
Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem is administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist. The 3 first patients infused (cohort 1a) received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. 2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage to a new low dose. (see section 1)). Approximately 3 patients will receive a lower dose of HepaStem (in cohort 1b) and approximately 15 patients will receive the higher dose in cohort 2 (cohort 2a + 2b + 2c + 2d). In total approximately 21 evaluable patients will be included. The total sample size will depend on the recommendations given by SMC in order to protect patient safety based on a risk assessment.

Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who received at least one infusion. All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, liver transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall.

	The first analysis will be performed after first 12 treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. A third analysis will be performed after all (approximately) 21 patients will have completed the 28 day active study period or have died or have been lost to follow-up. The fourth analysis will be performed after all (approximately) 21 patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The fifth and final analysis will be performed after these approximately 21 patients will have completed the 1 year follow-up or have died or have been lost to follow-up. The first study report will be produced after first 12 treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after all (approximately) 21 patients will have completed the 28 day active study period and 3 month follow-up or have died or have been lost to follow-up. The report will be updated after all approximately 21 patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Period	**Screening Period**	**Active period**							*Long term follow-up*			
	Baseline	**Treatment Period**					**Surveillance Period**					
Time	**Over 1-7 days prior D1**	Infusion D1	D4 ± 2 days	D8 ± 2 days	D12 ± 2 days	D14 ± 2 days	**D21** ± 2 days	**D28** ± 2 days	**M2** ± 2 weeks	**M3** ± 2 weeks	**M6** ± 2 weeks	**M12** ± 1 month
Informed Consent	X											
Eligibility criteria	X											
Demography & Medical History	X											
Physical exam	X	Xa	X	Xa	X	X	X	X	X	X	X	X
Vital Sign	X	Xb	X	Xb	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD, MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	X%	X	X%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	X%	X	X%	X	X	X	X	X	X	X	X
Lipase & Coagulation 2: C-protein, S-protein, Anti-Thrombin III	X											
Coagulation 1 : INR, aPTT	X	X+	X	X+	X	X	X	X	X	X	X	X
Virology status (HbS Ag, HCV, HEV, HIV), Aspergillosis test	X											
Coagulation 3 : Fibrinogen	X	X+		X+								
Coagulation 3 : D-Dimers, optional local TEG		X+		X+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	Xa		Xa		X		X				
TEG, TG	X	X*	X	X*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	Xa	X	Xa	X	X	X	X				X
Chest X-Ray	©											X
ECG	©											
Cardiac US Doppler	©	≠										X
Blood culture or other fluid culture	c											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶cells /kg body weight with a maximum of 25.10⁶cells + Hydrocortison (100mg) 15-30min prior infusion		X										
Cohort 2a & b : Infusion of 0.5.10⁶cells /kg body weight with a maximum of 50.10⁶cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2b only								
Cohort 2c & d : Infusion of 1.0.10⁶cells /kg body weight with a maximum of 100.10⁶cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2d only								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a: On infusion day: before infusion

b: On infusion day: before, during and after infusion

%: On infusion day: all parameters are measured prior infusion/platelets measurement to be performed prior and post infusion at 4h, 24h, 48h & 72h

C: Only if performed during the same admission

+: On infusion day: prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion

&: On infusion day: prior and 24h after infusion

©: if not already performed during same admission. If already performed, results collected

*: On infusion day: prior and 4h after infusion

AESI: Only Adverse Event of Special Interest to be reported

≠: cardiac US to be performed after infusion

1. BACKGROUND AND RATIONALE

1.1. CIRRHOSIS, ACUTE DECOMPENSATION AND ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Cirrhosis is a progressive chronic liver disease characterized by diffuse fibrosis, severe disruption of the intrahepatic venous flow, portal hypertension and liver failure. The course of cirrhosis is divided into two stages. Compensated cirrhosis defines the period between the onset of cirrhosis and the first major complication. During this period, which is relatively long in most patients (>10 years), symptoms are absent or minor, but liver lesions and portal pressure steadily progress. The term decompensated cirrhosis defines the period following the development of ascites (that is, the accumulation of large amounts of fluid within the peritoneal cavity), variceal haemorrhage and/or hepatic encephalopathy. This period is associated with short-term survival (3–5 years). It is increasingly evident that patients rarely die as a consequence of an end-stage irreversible destruction of the liver. Rather, in most patients, the cause of death is an acute deterioration in their chronic clinical condition promoted by a precipitating event — a syndrome termed acute-on-chronic liver failure (ACLF) (Arroyo et al. 2015).

It is of note that definitions on ACLF may differ worldwide. Given the heterogeneity and the importance of identifying patients four major societies/organisations have provided working definitions (APASL, NACSELD, WGO and EASL-CLIF). The common definition of ACLF is 'a syndrome characterised by acute decompensation of chronic liver disease associated with organ failure(s) and high short-term mortality'. According to the CLIF-ACLF definition developed based on the CANONIC study, ACLF is a recognised syndrome characterised by acute decompensation of cirrhosis associated with the failure of one or more organs and, in the more severe cases, system failure. The organs and systems most likely to fail are the liver, kidney, brain, coagulation, circulation and/or lungs. Patients have a high short term mortality of over 15 % at 28 days (Hernaez R et al, 2017). In the CANONIC study approximately 31% of patients admitted to a hospital for Acute Decompensation (AD) of cirrhosis had ACLF at admission (20%) or developed the syndrome during hospitalisation (11%). The common causes of acute decompensation of liver function included bacterial infections, alcoholic hepatitis, and gastrointestinal hemorrhages, but, in more than 40 % of patients, no precipitating event was identified (Moreau et al. 2013). Among patients with Acute Decompensation (AD), subgroups were identified as being at higher risk of progressing to full blown ACLF and thus at higher mortality risk (Arroyo et al. 2015).
Different grading/scoring systems have been developed in order to better determine prognosis and effectiveness of intervention and care. (Hernaez R et al, 2017).

In daily practice, MELD and Child Pugh scores are still strongly relied on to guide clinical care.

The Model for End-Stage Liver Disease, or MELD, is a scoring system for assessing the severity of chronic liver disease. This score is used by the United Network for Organ Sharing (UNOS) and Eurotransplant for prioritizing allocation of liver transplants. New MELD uses the patient's values for serum bilirubin, serum creatinine, sodium and the international normalized ratio for prothrombin time (INR) to predict survival.

Mortality and MELD score are linearly correlated amongst patients with end-stage liver disease listed for OLT with 3-month mortality estimated to be 4%, 27%, 76%, 83%, and 100% for MELD scores of <10, 10–19, 20–29, 30–39, and 40 or more respectively.

The Child–Pugh score is used in clinical practice to assess the prognosis of chronic liver disease, mainly cirrhosis. It was previously used for prioritizing allocation of liver transplants. The score employs five clinical measures of liver disease: total bilirubin, serum albumin, prothrombin time, ascites and hepatic encephalopathy. Each measure is scored 1–3, with 3 indicating most severe derangement. This leads to three Classes with one year overal survival of 100% for Class A, 81% for class B and 35% for class C. (see 0)

ACLF has been defined by the CLIF research consortium into four grades based on retrospectively fitting data on severity linked to mortality score (Moreau et al. 2013) (Table 1-1 and Table 1-2)

- ACLF grade 0 concerns 69.1 % of patients admitted to hospital with acute decompensation. The group is defined as no organ failure, single "non kidney" organ failure (ie, single failure of the liver, coagulation, circulation, or respiration) who had a serum creatinine level < 1.5 mg/dL and no hepatic encephalopathy, or as single cerebral failure with a serum creatinine level < 1.5 mg/dL. These patients have a 28-day and 90-day mortality rate of 4.7% and 14% respectively.
- ACLF grade 1 concerns 15.8 % of patients admitted to hospital with acute decompensation. The group is defined as single kidney failure or single non-kidney organ failure with an organ dysfunction (kidney or brain) and has a 28-day mortality rate of 23 %.
- Patients with ACLF grade 2, defined as two failing organs (10.9 % of patients admitted to hospital with acute decompensation) has an intermediate prognosis (28-day mortality rate of 31%).
- Finally, ACLF grade 3, defined as three or more organ failures (4.4 % of patients admitted to hospital with acute decompensation) has extremely high mortality rates, reaching 75 % after 28 days.

Among patients hospitalised with acute decompensation (AD) (pre ACLF according to the CLIF criteria but ACLF according to other classification systems), an analysis revealed five independent variables including age, serum sodium, white cell count, creatinine and INR as useful for defining a scoring system. The high-risk group (CLIF-C AD score > 60) and intermediate risk group (CLIF-C AD score 46-59) respectively have a 3-month mortality of over 30 % and between 2-30 %. The low risk AD group has a 3-month mortality below 2 % (Arroyo et al. 2015).

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years. The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

Cirrhotic patients with acute decompensation can only receive supportive treatments, such as antibiotics in case of infection, lactulose in case of encephalopathy, terlipressin and albumin in case of hepatorenal syndrome. However, at this moment, there are no treatments available to stop the inflammatory cascade often accompanying the acute decompensation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion,

which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 , renal replacement therapy
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may cause necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver transplant rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

Conclusion on patient population: Based on this information, Promethera Biosciences proposes that the patient population is defined as cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 µmol/L) and MELD < or = 35.

Patients should have coagulation parameters within the ranges below:

- Fibrinogen ≥ 80 mg/ dL
- Platelets ≥ 40.000/mm3

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidence supports the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properties of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells (similar cells to HHALPC) were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013).

Later, the technology of large-scale cell production was transferred to Promethera Biosciences where clinical batches of HHALPC are produced in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs).

Toxicology *in-vitro or in-vivo* studies aiming to demonstrate the safety, tolerability and tumorigenicity aspect of HepaStem were conducted. *In vivo* studies were performed in rats and mice. They included one study to assess the safety of the intravenous mode of administration. Two studies specifically assessed the risk of tumor formation as this risk has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. An *in vitro* study the pro-coagulant activity of HepaStem was confirmed (Please refer to the IB for more details).

In addition, *in vitro* studies show that HepaStem cells express variable immunomodulatory surface markers of interest and have immunomodulatory functional effects: HepaStem inhibits the proliferation of activated T-lymphocytes and blocks the maturation of monocytes (see Section 1.2.6). Furthermore, 6 *in-vivo* studies were conducted with HepaStem evaluating the immunomodulatory properties using the IV route of administration and mainly doses of 12.5 10^6 cells/kg. No safety signal was detected based on these *in vivo* studies.

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg) (4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did

undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. These data laid the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

Based on literature data and Promethera experiences, it can be concluded that liver-derived MSCs, including HepaStem have a pro-coagulant activity. This pro-coagulant activity is also expressed by other MSCs. The pro-coagulant activity might be linked to tissue factor expression, an activator of the coagulation cascade. The procoagulant effect could be modulated by the concomitant administration of bivaluridin during HepaStem infusion in UCD clinical trials in order to prevent, mainly, anticipated thrombotic events. Very high cell doses have been administered intra-portally in the UCD studies in which thrombotic events only occurred at high doses (range: 115 million to 4,1 billion total cells were administered in the portal vein as a split dose in 1 to 10 infusions spread over 1 to 4 consecutive days). Bivaluridin will not be used in the ACLF clinical study as its use has not been validated for late stage cirrhotic patients. Contrary to patients with urea-cycle disorders, coagulation disturbances are common in the late stage chronic cirrhosis population and are linked to liver insufficiency. (see 1.2.5)

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

In a first-in man cohort conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells (ADHLSC, similar cells to HHALPC) infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated this cell infusion well, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells

repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, spleen, right ankle, and spine, and had disappeared from the lungs. This is in line with the bio-distribution of another type of MSCs administrated in patients (BM-derived MSCs) that demonstrate a similar bio-distribution, with a first pass through the lung; within 24 hours, cells are mainly found in liver, spleen, kidneys and other inflamed areas, by 48 hours, more pronounced presence in the liver is observed. (NDS dossier remestemcel-L, Health Canada).

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For example, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012).

In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

It is clear from consultation of literature publications on cell therapy administration in advanced liver disease including decompensated liver cirrhosis and ACLF that doses of cell therapy protocols tended to be lower as compared to the normal range administered to patients in other immune-modulatory or anti-inflammatory protocols.

The doses and regimens administered to treat patients with chronic liver diseases, range from 0.03 over 0.5 to 1 million MSC cells/kg bodyweight. Different regimens were applied with repeated dosing up to 3 times for the lowest doses (0.03; 0,05 and 0,5 million cells/kg BW repeated 3 times). Most protocols administered the cell infusion intravenously although also other routes of administration were investigated such as intra-splenic, hepatic artery, intrahepatic, intra-lesional route of administration or central venous catheter into the femoral vein. (Berardis et al. 2015)

Based on the literature review, MSC administration is considered to be safe due to the lack of reports of significant adverse effects in the above studies, although a marked heterogeneity was observed among studies with regard to injection dose, frequency of injection, cell source, delivery route and study design. Most of these early studies reported improvements in liver function, ascites and encephalopathy.

In the first cohort of 3 patients in the HEP001 study the lowest dose (12.5×10^6 cells/kg) of the range of doses administered safely in previous studies of HepaStem in urea cycle disorder (UCD) and Crigler Najar pediatric patients was used to determine the dose in the HEP101 protocol. The (low) dose proposed (250

million cells/ infusion; ie. 3.5 10^6 cells/kg BW/infusion) was a reduction of 4x of the lowest dose tested previously (in the HEP001 protocol) and it was thought that it could be safely administered in cirrhotic patients. Additionally, the number of cells administered per infusion would be limited, similar to MSC doses given in immune mediated inflammatory diseases. In retrospect, it was clear that adaptation to the dose level similar to other MSCs given in immune-mediated inflammatory diseases was inadequate and did not take the specific case of severely ill chronic cirrhotic patients with acute decompensation into account.

Therefore, it seems that using a careful approach starting with doses commonly used in reported studies of decompensated cirrhosis and ACLF patients and published as being safe, appears to be an acceptable approach. Also, dose escalation to a maximum of 1.0 million cells/kg BW per infusion should be feasible based on a repetitive dosing schedule. In case of repetitive dosing, the doses will be given weekly, which allows time in case of fibrinolysis for the parameters to be corrected and return to normal.

1.2.6. Pre-clinical immunomodulatory data of liver-derived progenitor cells

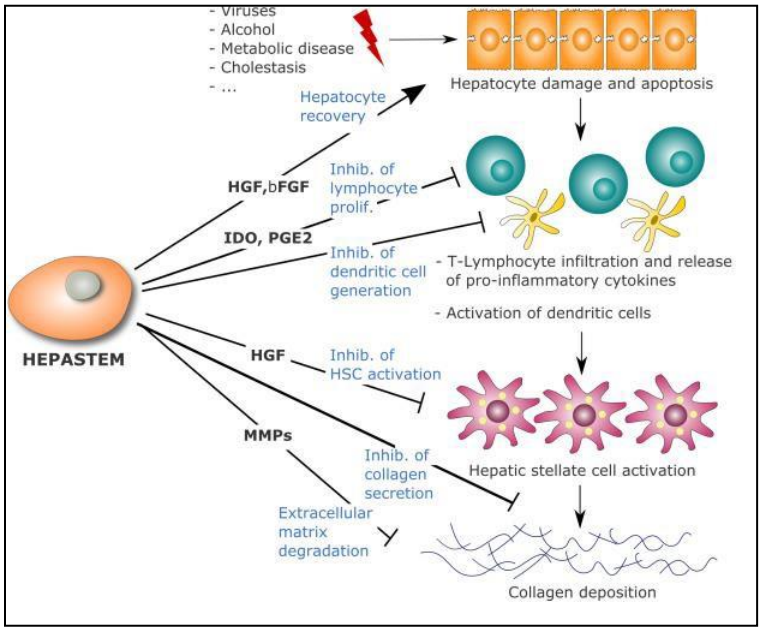
The first transcriptomics and secretomics tests performed on liver-derived progenitor cells (similar cells to HHALPC) grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (similar cells to HHALPC) (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature

dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibited. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models presents important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.7. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing to the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic

hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.8. Expected Benefits of HepaStem

Proposed mechanisms of action: after intravenous administration of HepaStem, cells are expected to circulate into the blood network where they can exert a systemic immunomodulatory action. At the same time, they have a main homing into the liver where they can thus also exert some important local immunomodulatory effects. They are expected to play their immunomodulatory roles through direct cell-to-cell interaction and through paracrine effects via the various cytokines, chemokines, MMPs and growth factors they may secrete. HepaStem could affect monocytes and DC recruitment, differentiation, maturation and function through cell contacts or paracrine signalling. HepaStem could also alter the proliferation and activation of T-lymphocytes that are another dysregulated cell type of the immune system in ACLF. In addition to modulate the behaviour of immune cells, HepaStem could modulate the proliferation and activation of hepatocytes and hepatic stellate cells and thus their secretory profiles, helping in this way the liver function recovery. The current *in vitro* and *in vivo* data, based on the scientific literature, and sponsor *in vitro* results, support all these potential immunomodulatory effects of HepaStem in ACLF patients.

Proposed clinical significant benefit: by these combined effects, HepaStem could play a favourable role in restoring an immunological balance in ACLF patients or patients at risk of ACLF, improving organ failure scores, improving clinical status, possibly leading to a resolution of this acute event and demonstrating improvement of transplantation free survival.

Considering the unmet medical need: i. the emergency to treat cirrhotic patients with Acute Decompensation (pre-ACLF or ACLF) due to the high mortality rate; ii. the shortage of healthy donors and the need of livers in the context of liver transplantation; iii. Concerns raised recently regarding artificial liver support; and iv. the mechanism of action of HepaStem, we can say that all these factors are in favour of a promising favourable benefit/risk balance for HepaStem. The exact profile of which patients will benefit most is under investigation, and also subject of this safety study.

1.2.9. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts with approximately 21 hospitalized patients in total. The study will include patients with acute decompensation of cirrhosis and with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 35, excluding patients with circulatory, respiratory failure or severe coagulations disorders. It is planned to have a first group of 6 patients approximately (cohort 1) being administered with the low dose.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days.

On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone or equivalent was given 15 to 30 min before each HepaStem infusion.

2 patients experienced an episode of severe bleeding. Therefore, it has been decided to reduce the dose in the low dose cohort to $0.25.10^6$ cells/kg bodyweight with a maximum of 25.10^6 cells in a single infusion. A reduction of minimum 10 times the dose previously used.

For cohort 1b: Approximately 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion

Once this has been proven safe, a second group of approximately 3 patients (cohort 2a) will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: Approximately 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

Additionally, approximately of 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort.

For cohort 2c: Approximately 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion.

For cohort 2d: Approximately 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).

HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period.

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 12 patients, appears to be an acceptable approach in patients with ACLF or with acute decompensation at risk of developing ACLF for whom no specific therapeutic or curative treatment exist. (See section 1.1)

HepaStem administration will be started rapidly after hospitalisation and will be completed within 1 day (cohort 1b, 2a, 2c) or within 1 week (cohort 2b, 2d). This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of patients with or at risk of developing ACLF. As ACLF and/or Acute Decompensation of cirrhosis is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up up to 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining the design of future efficacy clinical studies.

Study population

The patient population is defined by cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 35 (see section 1.1).

Patients should have coagulation parameters within the ranges below:

- Fibrinogen ≥ 80 mg/ dL
- Platelets ≥ 40.000/mm3

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort 1a - corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 14 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100

mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 28 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 million cells per infusion) is close to MSC doses given in other trials for immune-mediated inflammatory diseases (Section 1.2.5), therefore, it was expected to show a similar safety and efficacy profile. It corresponded also to the high dose of liver-dirived progenitor cells (similar cells to HHALPC) administered via IV to the heamophila patient (see 1.2.4).

Due to the severe bleeding that occurred in 2 of the 3 patients that received 250 10^6 cells (50 mL of HepaStem) per infusion, the next selected dose (low dose cohort 1b) will be reduced to $0.25.10^6$cells/kg bodyweight (with a maximum of 25.10^6 cells per infusion) administered in a single infusion (at least a 10x reduction of the dose administered in cohort 1a).

The second selected dose represents a two-fold increase from the dose in cohort 1b ($0.5.10^6$cells/kg bodyweight (with a maximum of 50.10^6 cells per infusion)

The third and fourth selected dose represents a two-fold increase from the cohort 2a and 2b. These dose still in the range of doses reported for MSCs and more in the range of doses administered in the specific case of severely ill chronic liver disease patients with ACLF and acute decompensation of cirrhosis. (see1.2.5)). (for additional information, please refer to the rationale for changes dated 28June2018).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 IU/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damage nor any respiratory symptoms, before homing mainly to the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF, it also can allow repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs also have a procoagulant activity comparable to liver-derived progenitor cells (similar cells to HHALPC) (Stephenne et al. 2012), nevertheless literature reports show that MSCs were safely administered by IV route without

anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015).

However, liver failure results in a state of “rebalanced hemostasis” marked by a decrease in both pro-coagulation and anticoagulation factors. Patients with severe liver disease are not auto-anticoagulated. In essence, patients with severe liver disease, acute and/or chronic, have a tenuous rebalanced hemostasis that is easily perturbed by various disease states and concomitant medications and invasive procedures. Bleeding events including severe forms are common in these end-stage liver disease patients. The events of epistaxis and bleeding from puncture sites that occurred in 2 patients in cohort 1a (in retrospect a high dose in late stage cirrhotic patients), have been recognised in the literature as case reports. It was also stated that epistaxis as an overlooked cause of massive haematemesis in cirrhosis should be added to the list of upper GI bleeding. (Johal et al 2003). Hence, cirrhotic patients including ACLF patients are at increased risk of bleeding or thrombosis. Therefore dose reduction from normal ranges applied in other immune-modulatory diseases, modification of inclusion criteria and increased surveillance of liver and coagualation parameters is indicated.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone or equivalent will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of different regimens of HepaStem in cirrhotic patients presenting with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of different dose regimens of HepaStem given in subsequent cohorts with 8(up to 21) hospitalized patients in total.

5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next patients enrolled will complete each dose cohort in a step wise approach and will receive a lower dose following SAEs observed in patient 2 and patient 3 in the cohort 1a (with high dose of cells).

Approximately three patients will be enrolled in cohort 1b.

Approximately fifteen other patients will be enrolled in cohort 2 (cohort 2a + 2b + 2c + 2d).

Approximately 3 patients of the cohort 2 (cohort 2a) will receive twice the dose compared to the cohort 1b.

Approximately 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

Additionally, approximately 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort.

The patients of the cohort 2c and 2d (approximately 3 patients for each subcohort) will receive up to 2 times the dose given in to the cohort 2b.

The study will recruit patients who are hospitalized for Acute Decompensation of cirrhosis and/or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

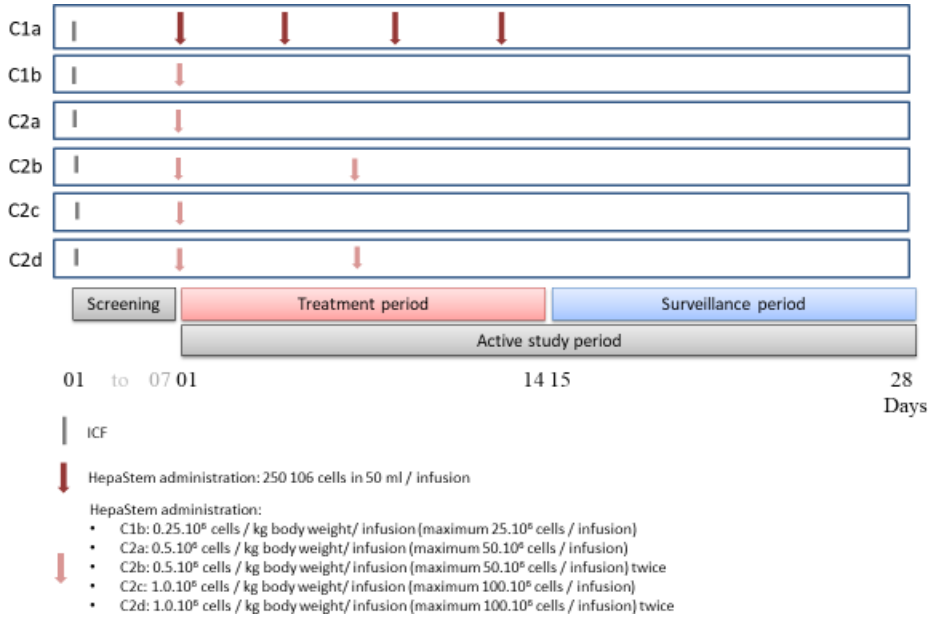
Screening period: Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days). The duration of the screening period plus the active period will last up to 35 days (± 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



Planned schedule:

For cohort 1a, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (±2 days); at least a 2-day interval without infusion must be respected between infusion days.

In cohort 1a, 250 million cells in 50 ml were administrated on each infusion day, leading to a total of 1 billion cells if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before each HepaStem infusion.

Actual schedule:

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone or equivalent was given 15 to 30 min before each HepaStem infusion.

Planned schedule:

For cohort 1b: Approximately 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion

For cohort 2a: Approximately 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: Approximately 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

Additionally, approximately 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort.

For cohort 2c: Approximately 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion.

For cohort 2d: Approximately 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).

Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of external members and Promethera members (See Section 9.13):

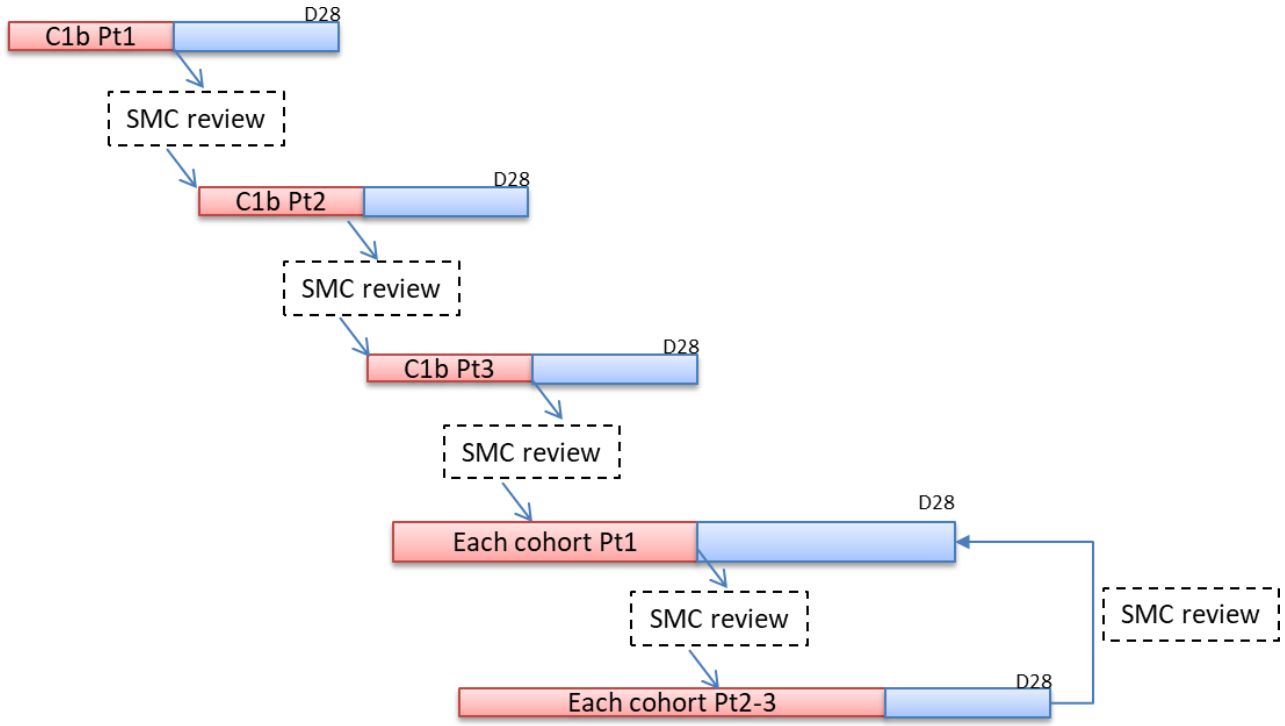
As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a, 2b, 2c and 2d), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patients parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a single administration of $0.5.10^6$ cells/kg body weight).

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring committee



The study assessments are described in Section 6.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the safety follow-up period.

After completion of this study, patients will be followed-up in the Patient Long term Follow up Registry for 5 years.

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
  - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome of liver transplantation
- New ACLF episode will be summarized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in up to 25 centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (35 days (± 2 days) in the screening plus active treatment period), and thereafter for an additional period up to 1 year post first HepaStem infusion (D1) in the safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Cirrhotic patients with Acute Decompensation at risk of developing ACLF at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining signed informed consent, the screening period may last maximum 7 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 years old.
2. Signed Informed Consent.
   N.B: In case of hepatic encephalopathy, if the patient is not able to understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.
3. Cirrhosis as diagnosed by
   - liver histology or
   - clinical and imaging examination (may include fibroscan).
4. Patient with Acute Decompensation of cirrhosis
5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Thrombosis of the portal vein.
2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.

   Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.
3. Ongoing uncontrolled bleeding.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.

5. Clinical evidence of Aspergilus infection.
6. Circulatory failure defined by inability to maintain a mean Blood pressure ≥ 70 despite use of vasopressors
7. Mechanical ventilation due to respiratory failure.
8. Coagulation disorders defined as:
    - Fibrinogen < 80 mg/dL
    - Platelets < 40.000/mm^3
9. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator.
10. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.
11. MELD score > 35.
12. Previous organ transplantation and/or ongoing immunosuppressive treatments.
13. Postoperative-decompensation following hepatectomy.
14. Renal failure due to chronic kidney disease.
15. Clinically significant left-right cardiac shunt.
16. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
17. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. In case of suspicion of HCC, all exam should be done to confirm or not the diagnosis prior enrolment.
18. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
19. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
20. Participation to any other interventional study within the last 4 weeks.
21. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration are the following:

- **Transitory discontinuation:** Coagulation disorders considered as significant (Fibrinogen <80 mg/dL, or Platelets < 40.000/mm^3) by the PI prior to each infusion should preclude the administration of Hepastem.

- Absence of portal vein flow that are in favor of a thrombosis of the portal vein prior to the infusion should preclude the administration of Hepastem.
- Transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.

Infusions may be restarted after recovery. If planned infusions are not performed within treatment period (± 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAWAL CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum - 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).
- The exact dosage (volume) of Hepastem infused to the patient will be calculated based on the weight of the patient on the day of infusion (0.25.10^6 cells per kg bodyweight with a maximum of 25.10^6 cells/infusion (5 mL) for cohort 1b,0.5.10^6 or 1.0.10^6.cells per kg bodyweight with a maximum of 50.10^6 or 100. 10^6 cells/infusion (10 mL or 20mL) for cohort 2.)
- As the exact volume to infused can be low (depending on the patient's weight), it is recommended to flush after the infusion physiological solution (NaCl 0,9%) to ensure that all the product is infused.

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and the syringe will

be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused.

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given.

For cohort 1b: Approximately 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells per infusion). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapated accordingly. A single infusion is expected to last minimum 5 min (for 5 mL of reconstituted Hepastem).

For cohort 2a and 2b: Approximately 3 patients will receive HepaStem in a single infusion (cohort 2a) and approximately 6 patients in 2 repeated infusions one week apart (cohort 2b). The dosage of Hepastem per infusion will be $0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion.

For cohort 2c and 2d: Approximately 3 patients will receive HepaStem in a single infusion (cohort 2c) or in 2 repeated infusions one week apart (cohort 2d). The dosage of Hepastem per infusion will be $1.0.10^6$ cells per kg body weight with a maximum of 100.10^6 cells per infusion).

Each infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single infusion is expected to last maximum 20 min (for 20 mL of reconstituted Hepastem).

The full procedure describing how to adapt the volume of HepaStem to be administrated to the patient's body weight is in the HepaStem Manual.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate, ICUs or standard units. Patients will be hospitalised during HepaStem treatment period to allow a continuous monitoring of the patient.

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension has to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation : the syringe has to be inversed 10 times every 3 minutes.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics, risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), and risks observed with the infusion of HepaStem in the cohort 1a, main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis or to consumption of coagulation factors and subsequent bleeding; respiratory disorder as cells first transit to the lungs; hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the risks mentioned below, there might be other, at this time, unknown risks.

5.4.1. Risk and Benefit assessment

ACLF patients or patients at high risk to develop ALCF are at high mortality risk and there is currently no specific treatment for these patients. Orthotopic liver transplantation is often not a possible option for these patients. By its potential combined effects, HepaStem could play a favourable role in restoring an immunological balance in pre-ACLF / ACLF patients, leading to a resolution of this acute event and showing improvement of organ function and transplantation free survival. The main identified risks linked to HepaStem are activation of the coagulation cascade and may lead to thrombosis (observed in UCD patients) or bleeding (observed in ACLF patients). The safety measures described below (see section 5.5) are recommended to minimize the risks of the administration of HepaStem in ACLF or pre-ACLF patients at high risk of short term mortality.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients.

Before each infusion, the investigator will have to make sure the patient has the minimum criteria to receive HepaStem (see 4.4 - Criteria for study treatment discontinuation).

Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lung disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect.

Number of cells administered for the cohort 1a, 2a,2b, 2c and 2d per infusion will be maximum 25.10^6 cells (cohort 1a),50.10^6 cells (cohorts 2a and 2b) or 100.10^6 cells (cohorts 2c and 2d) and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min. The lower dose regimen will be applied before the higher one. These doses are in the very low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication and re-adjusted to the range of MSC doses given in decompensated cirrhotic and ACLF patients (See Section 1).

Furthermore, **the coagulation parameters will be closely monitored** prior and after the infusion process at 4h, 8h, 12h, 24h, 48h and 72h post infusion. (Including INR, aPTT, fibrinogen, D-Dimers, coagulation factors (pre and 24h post infusion), and TEG (optional, only if measurement can be done locally and up to investigator's judgment)

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion, and patient with risk of bleeding (defined by recent major invasive procedure, non controlled gastrointestinal hemorrhage and/or coagulation disorders) will be excluded from the study.

In case major changes in the coagulation parameters and/or clinically significant bleedings suggestive of important coagulation factors consumption occur, according to the investigator's judgement, it could be envisioned to administer coagulation factors in the form of fresh frozen plasma (FFP), fibrinogen concentrate (ie RiaSTAP), and/or antifibrinolytics (ie tranexamic acid). (cfr. both study patients in cohort 1a responded well to treatment with FFP and/or addition of coagulation factors).

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions.

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours. Portal patency can also be investigated with abdominal CT scan or MRI. **Hepastem will be administred only if portal vein patency is demonstrated.**

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone or equivalent will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no

tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Safety follow-up period up to 1 year after D1 (first HepaStem infusion).

Thereafter, patients will be invited to be followed in the Patient long term safety follow up Registry for 5 additionnal years.

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before the HepaStem infusion.

All efforts should be made to use 100 mg of Hydrocortisone according to the above-mentioned protocol. Nevertheless, if 100 mg hydrocortisone is not available, the following equivalent can be accepted:

Compound	***Equivalent Dose***	***Biological Half-life***
Cortisone	125 mg	Short (8-12 hours)
Hydrocortisone	100 mg	Short (8-12 hours)
Prednisolone	25 mg	Intermediate (12-36 hours)
Methylprednisolone	20 mg	Intermediate (12-36 hours)

The exact treatment administrated should be documented in the Source Document and in the eCRF.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and and treatment period, patients will be hospitalised. Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1

On HepaStem infusion days, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, coagulation factors, TEG (if already performed as part of the clinical routine and up to investigator's judgment) a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

The careful evaluation of these parameters will allow or not the infusion (see section 4.4 – Criteria for treatment discontinuation).

On the other days during the hospital stay, patients will be followed-up according to usual practice.

A study visit will be performed on Day 14 $\pm$ 2 days, including the evaluations listed below.

After the treatment period, study visits will be done on days 21 and 28 ($\pm$ 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 ($\pm$ 2 weeks), Month 3 ($\pm$ 2 weeks), Month 6 ($\pm$ 2 weeks), and Year 1 ($\pm$ 1 month).

Up to the 28 days visit, all SAEs will be collected. After the 28 days visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be included in the patient long term safety follow up Registry for 5 additionnal years.

6.2.1. Study assessments

- All AEs up to Day 28
- All AESI up to 1 Year.
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
    - GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
    - INR
    - aPTT
    - fibrinogen
    - D-Dimers
    - TEG (optional, only if measurement can be done locally and up to investigator's judgment)
- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion.
- Lipase: at screening
- Fibrinogen at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening

- Protein C, Protein S, anti-thrombin III: at screening. Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2), 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also on M12
- Chest x-ray at screening (if not performed during same admission) and at M12,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)
- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

<u>Thromboelastogram and thromboglobulin generation tests</u>

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min twice). The plasma will be collected and put in cryotubes (min. 2 tubes containing each min. 500 µL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Mounier 53,
1200 Woluwe-Saint Lambert BELGIUM

<u>Anti-HLA antibodies</u>

Serum will be collected and send within 48 hours (ambient temperature) to :

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Mounier 53 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHARTS

Table 6-1 Study Flowchart

Period	**Screening Period**	**Active period**							*Long term follow-up*			
	Baseline	**Treatment Period**					**Surveillance Period**					
Time	**Over 1-7 days prior D1**	Infusion D1	D4 ± 2 days	D8 ± 2 days	D12 ± 2 days	D14 ± 2 days	**D21** ± 2 days	**D28** ± 2 days	**M2** ± 2 weeks	**M3** ± 2 weeks	**M6** ± 2 weeks	**M12** ± 1 month
Informed Consent	X											
Eligibility criteria	X											
Demography & Medical History	X											
Physical exam	X	Xa	X	Xa	X	X	X	X	X	X	X	X
Vital Sign	X	Xb	X	Xb	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD, MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	X%	X	X%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	X%	X	X%	X	X	X	X	X	X	X	X
Lipase & Coagulation 2: C-protein, S-protein, Anti-Thrombin III	X											
Coagulation 1 : INR, aPTT	X	X+	X	X+	X	X	X	X	X	X	X	X
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen	X	X+		X+								
Coagulation 3 : D-Dimers, optional local TEG		X+		X+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	Xa		Xa		X		X				
TEG, TG	X	X*	X	X*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	Xa	X	Xa	X	X	X	X				X
Chest X-Ray	©											X
ECG	©											
Cardiac US Doppler	©	≠										X
Blood culture or other fluid culture	c											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶ cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X										
Cohort 2a & b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2b only								
Cohort 2c & d : Infusion of 1.0.10⁶ cells /kg body weight with a maximum of 100.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2d only								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a: On infusion day: before infusion	b: On infusion day: before, during and after infusion
%: On infusion day: all parameters are measured prior infusion/platelets measurement to be performed prior and post infusion at 4h, 24h, 48h & 72h	C: Only if performed during the same admission
+: On infusion day: prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion	&: On infusion day: prior and 24h after infusion
©: if not already performed during same admission. If already performed, results collected	*: On infusion day: prior and 4h after infusion
AESI: Only Adverse Event of Special Interest to be reported	≠: cardiac US to be performed after infusion

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

Untill visit day 28 inclusive, all AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Adverse Event of Special Interest are defined as:

- AEs with fatal outcome

- Liver Transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Untill visit day 28 inclusive, any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

Untill visit day 28 inclusive, all SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possible, probable or definite.

7.1.5. Serious Adverse Drug Reactions (SADR)

A SADR is any ADR that is serious as per the above criteria.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

<u>Possibly</u>

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probably

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definitly

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

Moderate, when interfering with ADL;

Severe, when impeding ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

7.2.5. Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report. AESI Reporting Procedures

Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SADR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts each with approximately 21 hospitalized patients in total.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

The 3 first patients infused (cohort 1a) : 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone or equivalent was given 15 to 30 min before each HepaStem infusion.

2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage (see section 1).

Approximately 3 patients will receive a lower dose of HepaStem (in cohort 1b) and approximately 15 patients will receive the higher dose in cohort 2 (cohort 2a + 2b + 2c + 2d).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Since it is a safety study, any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

In total approximately 21 evaluable patients will be included. The total sample size will depend on the recommendations given by SMC in order to protect patient safety based on a risk assessment.

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied.

Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (AE with fatal outcome, liver transplantation, onset of malignancies, hospitalization for ACLF, AEs assessed by the investigator as possibly related to HepaStem) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after first 12 treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

A third analysis will be performed after all (approximately) 21 patients will have completed the 28 day active study period or have died or have been lost to follow-up.

The fourth analysis will be performed after all (approximately) 21 patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The fifth and final analysis will be performed after these approximately 21 patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after first 12 treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after all (approximately) 21 patients will have completed the 28 day active study period and 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after all approximately 21 patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], if the patient is not able to understand the study based on the investigator's judgment, the informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant

protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences

must “verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection”.

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator’s Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient’s further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each major protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of members, all external and independent to Promethera. The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.

2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.

As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a,2b, 2c and 2d), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme of premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patient's parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a unique administration of $0.5.10^6$ cells/kg body weight).

5. The SMC will review severe coagulation events assessed as related to HepaStem administration by the investigator.
6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- One or more biostatisticians

- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists and Promethera Biosciences members may provide additional information if additional expertise is desired, but are not members of the SMC.

The frequency of SMC meetings will depend on several factors including the rate of enrollment, completion of patients in the dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL, renal replacement therapy
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	 >300 >357	 ≤300 - > 200 >214- ≤357	 ≤200 ≤214

Arroyo et al. 2015

11.2. CLIF ACLF GRADE

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

11.3. CLIF-C ACLF SCORE

CLIF-C ACLF = 10 × [(0,33 × CLIF OF + 0.04 × Age + 0,63 × Ln(WBC{10^9 cells/L}) – 2]

11.4. CLIF CONSORTIUM ACUTE DECOMPENSATION SCORE (CLIF-C AD)

CLIF-C AD = 10 × [(0,03 × Age {years} + 0,66 × Ln(Creatinine{mg/dL} + 1.71 × Ln(INR) + 0,88 × Ln(WBC{10^9 cells/L}) – 0,05 × Sodium {mmol/L} + 8]

Jalan et al. 2015

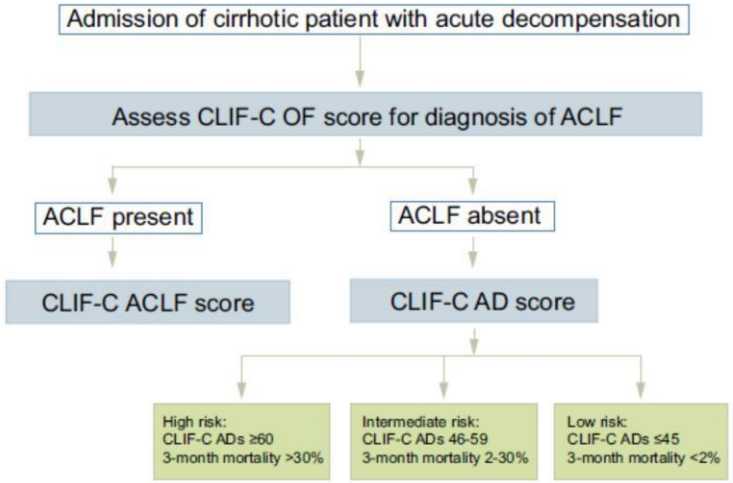

Fig. 4. Algorithm for the sequential use of the EASL-CLIF Consortium predictive scores in patients with cirrhosis admitted to hospital with acute decompensation.

11.5. MELD SCORE

MELD score is calculated using serum bilirubin, serum creatinine, and International Normalized Ratio (INR) and is given by the formula :

MELD(i) = (0.957 * ln(Serum Cr) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10 (if hemodialysis, value for Creatinine is automatically set to 4.0)

MELD Score (2016) = MELD(i) + 1.32*(137-Na) – [0.033*MELD(i)*(137-Na)]

Note: Sodium has a range of 125-137 mEq/L

The score can be calculated using online website https://www.mdcalc.com/meld-score-model-end-stage-liver-disease-12-older

11.6. CHILD PUGH SCORE

Measure	1 point	2 points	3 points
Total bilirubin, μmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8

Prothrombin time prolongation (s) Or INR	<4.0 <1.7	4.0–6.0 1.7-2.3	> 6.0 >2.3
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	Class	One year survival	Two year survival
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

Singal AK, Kamath PS. Model for End-stage Liver Disease. J Clin Exp Hepatol. 2013 Mar;3(1):50-60. Review.

11.7. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 14 December 2018

Version Number: 6.0

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____ _____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 14 December 2018

Version Number: 6.0

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competent Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

Silver Ocean Ventures SAS, CEO, represented by John Tchelingerian Promethera Biosciences	Date
Etienne Sokal, Chief Scientific & Medical Officer Promethera Biosciences	Date

Clinical Study Protocol

EudraCT 2016-001177-32

Protocol Code: HEP101

Version 6.1 – 05 February 2019

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

STUDY SPONSOR	**PROMETHERA BIOSCIENCES** *Rue Granbonpré 11* *B-1348 Mont Saint Guibert, Belgium*
COORDINATING INVESTIGATOR	Prof. Dr. Frederik Nevens, University Hospitals KU Leuven, Belgium
STUDY COMMITTEE	Etienne Sokal, MD - Chief Scientific & Medical Officer John Tchelingerian, PhD – Chief Executive Officer
MONITORING & STUDY COORDINATION	Virginie Barthel, T.+32.471.784.097 Nele Vanparys, T.+34.665.245.704
SAFETY & PHARMACOVIGILANCE	pv@promethera.com **T.*+32.10.39.43.43***

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LIST OF ABBREVIATIONS

AD	Acute Decompensation
ADR	Adverse Drug Reaction
AE	Adverse Event
APTT	Activated Partial Thromboplastin Time
ATMP	Advanced Therapy Medicinal Product
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Competent Authorities
cc	cubic centimeter
Cl	Chloride
cm	Centimeter
CN	Crigler-Najjar
eCRF	electronic Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DLP	Data Lock Point
DMSO	DiMethyl SulfOxide
DNA	DeoxyriboNucleic Acid
DP	Drug Product
DSUR	Development Safety Update Report
EDTA	EthyleneDiamineTetraAcetic acid
EMA	European Medicines Agency
EBV	Epstein Barr Virus
EU	European Union
EVCTM	EudraVigilance Clinical Trial Module
FDIS	Final Draft International Standard
FU	Follow-up
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
γGT	γ Glutamyl Transferase
H, hrs	Hour, hours
Hct	Hematocrit
HE	Hepatic Encephalopathy
Hgb	Hemoglobin
HHALPC	Heterologous Human Adult Liver-derived Progenitor Cells
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation

IEC	Independent Ethics Committee
IFU	Instructions For Use
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISO	International Organization for Standardization
IV	IntraVenous
K	Potassium
kg	Kilogram
LCT	Liver Cell Transplantation
LDH	Lactate DeHydrogenase
LL	Lower Limit
LMWH	Low Molecular Weight Heparin
M	Month
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
min	Minute
ml	Milliliter
MSCs	Mesenchymal Stem Cells
MVR	Monitoring Visit Report
Na	Sodium
NCI	National Cancer Institute
NH_3	Ammonia
OLT	Orthotopic Liver Transplantation
PB	Promethera Biosciences
PCA	Pro-Coagulant Activity
PEDI	Laboratoire d'Hépatologie Pédiatrique
PT	Prothrombin Time
RBC	Red Blood Cell
RT-PCR	Real Time Polymerase Chain Reaction
s	Seconds
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SADR	Serious Adverse Drug Reaction
SGOT **SGPT**	Serum Glutamic Oxaloacetic Transaminase Serum Glutamate Pyruvate Transaminase
SIV	Site Initiation Visit
SMC	Safety Monitoring Committee
SMT	Standard Medical Treatment
SPC	Summary of Product Characteristics

SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
TF	Tissue Factor
TT	Thrombin time
UCD	Urea Cycle Disorders
UCL	Université Catholique de Louvain
UL	Upper Limit
US	UltraSound
UV	UltraViolet
V	Visit
WBC	White Blood Cell

Study Synopsis

Study Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure
EudraCT & Protocol code	Protocol n° HEP101 / EudraCT 2016-001177-32
Protocol version and date	Version 6.1 - 05 Feb 2019
Indication	Acute on Chronic Liver Failure (ACLF)
Study Phase	II
Sponsor	PROMETHERA BIOSCIENCES
Investigational product	HepaStem: Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC).
Objective	PRIMARY OBJECTIVE: To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period. SECONDARY OBJECTIVES: Preliminary efficacy: To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion. Long term safety: To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.
Number of Patients	Approximately twenty-one (21) evaluable Patients
Number of Centers	Up to 25 European Centers
Study Design	Interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts with 18 (approximately 21) hospitalized patients in total 5 patients were screened in the cohort 1a3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 ce
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 ce
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 ce

The next patients enrolled will complete each dose cohort in a step wise approach and will receive a lower dose following SAEs observed in patient 2 and patient 3 in the cohort 1a (with high dose of cells)

Approximately three patients will be enrolled in cohort 1b.

Approximately fifteenother patients will be enrolled in cohort 2 (cohorts 2a + 2b + 2c + 2d)

Approximately 3 patients of cohort 2a will receive twice the dose compared to cohort 1b.

Approximately 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

Additionnally, approximately 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort.

The patients of the cohort 2c and 2d (approximately 3 patients for each subcohort) will receive up to 2 times the dose given in to the cohort 2b

The statistical analysis will take into consideration the different doses applied.

Study periods

The study will recruit cirrhotic patients who are hospitalized for ACLF or Acute Decompensation at risk of developing ACLF

Patients will be recruited at hospitals with specialized hepatology and intensive care units. Patients with Acute Decompensation of cirrhosis at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last maximum 7 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

	The study is divided in the following periods: screening period, active study period divided in treatment period and evaluation period, and long-term safety follow-up. Screening period: Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. Active study period: The active study period will last 28 days (± 2 days). The duration of the screening period plus the active period will last up to 35 days (± 2 days). Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient. Various dose regimens of HepaStem will be given, which differ in the amount of cells per infusion and/or in the number of infusions. The 3 patients in the cohort 1a received the dose regimen of 250.10^6 cells per infusion – this represents approximately 2.9 to $3.4.10^6$ cells/kg bodyweight in the first cohort. (cohort 1a) Approximately three patients in cohort 1bwill receive a lower dose (minimum ten times lower) in a single infusion ($0.25.10^6$ cells /kg bodyweight with a maximum of 25.10^6 cells per infusion). Approximately 3 patients in cohort 2 (cohort 2a) will receive twice the dose of the patients in cohort 1b ($0.5.10^6$ cells/kg bodyweight with a maximum of 50.10^6 cells per infusion). Approximately 3 patients in cohort 2 (cohort 2b) will receive up to 2 doses of $0.5.10^6$ cells/ kg bodyweight 1 week apart ($0.5.10^6$ cells/kg bodyweight per infusion with a maximum of 50.10^6 cells per infusion). Additionnaly, approximately 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort. Approximately 3 patients in cohort 2 (cohort 2c) will receive twice the dose of the patients in cohort 2a (1.10^6 cells/kg bodyweight with a maximum of 100.10^6 cells per infusion). Approximately 3 patients in cohort 2 (cohort 2d) will receive up to 2 doses of 1.10^6 cells/ kg bodyweight 1 week apart (1.10^6 cells/kg bodyweight per infusion with a maximum of 100.10^6 cells per infusion).

	Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of members, all external and independant to Promethera Biosciences. As a minimum, the safety data will be reviewed by the SMC at the following timepoints : - For each cohort (1b, 2a,2b, 2c and 2d), when the first evaluable patient has received the HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients. - For the first cohort (1b), when the second evaluable patient has received HepaStem infusion (complete scheme or premature stop), the patient data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients. - When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patients in the next cohort. In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized. If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient. More specifically, based on the patients' parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg bodyweight) instead of a single administration of $0.5.10^6$ cells/kg bodyweight). Furthermore, the 3 patients of each cohort will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete sheme or premature stop). <u>Long-term safety follow-up</u>: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion (D1) in the safety follow-up period. After completion of this study, patients will be invited to be followed-up in the Patient long-term safety follow-up Registry for 5 additionnal years.

Study duration	The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (35 days (± 2 days) in the screening plus active treatment period), and thereafter for an additional period up to 1 year post first HepaStem infusion (D1) in the safety follow-up.
Study Treatments	HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at $50x10^6$ cells/ml equivalent to $250x10^6$ cells per vial. Prior to administration, the cells will be reconstituted with 45 ml of diluent. In cohort 1a, 50 ml was given per infusion. For cohorts 1b, 2a,2b, 2c and 2d the volume of HepaStem administered will be adapted to the patient's bodyweight.
Treatment Schedule and Dosage Regimen	All patients will receive standard medical treatment (SMT) as required by their clinical status. HepaStem will be infused intravenously through a peripheral catheter or through a central line, up to the choice of the investigator based on the patient's status. The infusion rate shall not exceed 1 ml per min. For cohort 1, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion had to be respected between infusion days. The *Planned* schedule was: in cohort 1a, 250 million cells in 50 ml were administered on each infusion day, leading to a total of 1 billion cells, if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone was expected to be given 15 to 30 min before each HepaStem infusion. The *Actual* schedule is: in cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. For cohort 1b: Approximately 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. For cohort 2a: Approximately 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of

	maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent (see section 5.6 'Concomitant treatments') will be given 15 to 30 min before HepaStem infusion. For cohort 2b: Approximately 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation). Additionnaly, approximately 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort. For cohort 2c: approximately 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. For cohort 2d: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).
Eligibility – Inclusion criteria	<u>Inclusion criteria</u>: 1. Adult aged between 18 and 70 years old. 2. Informed Consent. N.B: In case of hepatic encephalopathy, if the patient is not able to understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

	3. Cirrhosis as diagnosed by (1) liver histology or (2) by clinical and imaging examination (may include fibroscan). 4. Patient with Acute Decompensation of cirrhosis 5. Serum total Bilirubin ≥ 6 mg/dL (≥100 umol/L)
Eligibility - Exclusion Criteria	<u>Exclusion criteria</u>: 1. Thombosis of the portal vein. 2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion. 3. Ongoing uncontrolled bleeding. 4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h. 5. Clinical evidence of Aspergillus infection. 6. Circulatory failure defined by inability to maintain a mean Blood pressure ≥ 70 despite use of vasopressors 7. Mechanical ventilation due to respiratory failure 8. Coagulation disorders defined as: • Fibrinogen < 80 mg/dL • Platelets < 40.000/mm^3 9. Major invasive procedure within 1 week before the infusion (including but not limited to transjugular liver biopsy) 10. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period. 11. MELD score > 35. 12. Previous organ transplantation and/or ongoing immunosuppressive treatments. 13. Postoperative-decompensation following hepatectomy. 14. Renal failure due to chronic kidney disease. 15. Clinically significant left-right cardiac shunt. 16. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.

	17. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. In case of suspicion of HCC, all exam should be done to confirm or not the diagnosis prior enrolment. 18. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment. 19. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study. 20. Participation to any other interventional study within the last 4 weeks. 21. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.
Study Endpoints	<u>Primary endpoint</u>: Safety • AEs reported up to Day 28 of the active study period, assessed for serisousness, severity, relationship to IMP and/or IMP administration procedure The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28. The relationship will be assessed based on investigator assessment, and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline. <u>Secondary Endpoints</u>: • Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Mortality ○ Liver transplantation ○ Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score, Child Pugh score. • Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1 ○ Bilirubin, creatinine, INR and albumin values • Long term safety follow-up ○ Adverse Events of Special Interest (AESIs) will be summarized at Month 3 and Year 1 ▪ SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of the transplantation, New ACLF episode will be summarized at Month 3 and Year 1
Study Assessment visits	<u>Study visits</u> During the screening and treatment period, patients will be hospitalised.

	During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit). Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility. Patients will be treated in a stepwise approach as described in Section 3.1. On HepaStem infusion days, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, TEG (if already performed as part of the clinical routine and up to investigator's judgment), coagulation factors (intrinsic and extrincsic pathway), a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day (even if HepaStem infusions are prematurily stopped). The careful evaluation of these parameters will allow or will not allow the infusion (see section 4.4 – Criteria for treatment discontinuation). On the other days during the hospital stay, patients will be followed-up according to usual practice. A study visit will be performed on Day 4, 8, 12 and 14 ± 2 days post 1st infusion, including the evaluations listed below. After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged. After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month). Up to Day 28 visit, all SAEs will be collected. After Day 28 visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with

	fatal outcome, transplantation and outcome of the transplantation, malignancies, new AD and/or ACLF episode, AEs assessed by the investigator as possibly related to HepaStem (see Section 7.1.2).
	
	At the Month 12 study visit, patients will be invited to be included in the Patient long-term follow-up registry.
	
	<u>Study assessments</u>
	
	• All AEs up to Day 28
	• All AESI up to 1 Year.
	• Concomitant medication modifications up to Day 28
	• Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	• Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation):
	  ○ At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ Before, during and after infusion
	• West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD score (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3. 6 and 12
	• Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
	  ○ White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
	  ○ GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
	  ○ Creatinine, Urea or BUN
	  ○ CRP
	  ○ INR, aPTT
	  ○ Serum albumin, sodium, potassium
	• Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
	  ○ INR
	  ○ aPTT
	  ○ fibrinogen

	○ D-Dimers ○ TEG (optional, only if measurement can be done locally and up to investigator's judgment) • Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion. • Lipase: at screening • Fibrinogen at screening • Viral serology (HIV, HCV, HEV, HbS antigen) and Aspergilus detection: at screening (if not performed during same admission) • Urine test (Sediment, Creat, Glc, Protein, Albm): at screening • Protein C, Protein S, anti-thrombin III: at screening. In case of deterioration of the coagulation, these measurements will be repeated. • Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2b), 12, 14, 21, 28 (blood testing in central lab) • Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab) • Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab) • Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also at M12.Chest x-ray : at screening (if not performed during same admission) and at M12, • Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected) • ECG: at screening (if not performed during same admission). Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12. Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion. In case of premature withdrawal from study, an end of study visit should be perfomed if possible at the time of study withdrawal. In case of liver transplantation during the course of the study, a sample of the explanted liver will be collected if possible.
Prohibited Medications and Food	Patients are requested to accept abstinence from alcohol during the active study period (Day 28).

Sample Size Considerations	The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem is administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting in a cohort of 6 patients with a dose regimen close to those reported as being safe in MSC clinical trials, then testing in a second cohort of 6 patients a higher dosage also reported as safe appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist. The 3 first patients infused (cohort 1a) received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion. 2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage to a new low dose. (see section 1)). Approximately 3 patients will receive a lower dose of HepaStem (in cohort 1b) and approximately 15patients (new high dose cohort) in cohort 2 (cohort 2a + 2b + 2c + 2d). In total approximately 21 evaluable patients will be included. The total sample size will depend on the recommendations given by SMC in order to protect patient safety based on a risk assessment
Analytical Methods	Being a safety study also exploring efficacy, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied. Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category. Missing values will not be imputed. All statistical analyses will be based on the safety population defined as the subset of included patients who received at least one infusion. All study variables will be listed by treatment dose and overall. AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications

	and treatments will be coded using the World Health Organization (WHO) Drug Dictionary. Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall. All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs. AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance. Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported. Adverse Events of special interest (SAE with fatal outcome, transplantation and outcome of the transplantation, onset of malignancies, new ACLF episodes) will be listed and summarised by treatment dose and overall. The first analysis will be performed after treated patients of cohorts 1 and 2 will have completed the 28 days active study period or have died or have been lost to follow-up. The second analysis will be performed after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. A third analysis will be performed after all (approximately) 21 patients will have completed the 28 day active study period or have died or have been lost to follow-up. The fourth analysis will be performed after all (approximately) 21 patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The fifth and final analysis will be performed after these approximately 21 patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

	The first study report will be produced after first 12 treated patients of cohorts 1 and 2 will have completed the 28 days active study period or have died or have been lost to follow-up. The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up. The report will be updated after all (approximately) 21 patients will have completed the 28-day active study period and 3 month follow-up or have died or have been lost to follow-up. The report will be updated after all approximately 21 patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

Period	**Screening Period**	**Active period**										
	Baseline	**Treatment Period**					**Surveillance Period**		***Long term follow-up***			
Time	**Over 1-7 days prior D1**	Infusion D1	D4 ± 2 days	D8 ± 2 days	D12 ± 2 days	D14 ± 2 days	**D21** ± 2 days	**D28** ± 2 days	**M2** ± 2 weeks	**M3** ± 2 weeks	**M6** ± 2 weeks	**M12** ± 1 month
Informed Consent	X											
Eligibility criteria	X											
Demography & Medical History	X											
Physical exam	X	Xa	X	Xa	X	X	X	X	X	X	X	X
Vital Sign	X	Xb	X	Xb	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD, MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	X%	X	X%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	X%	X	X%	X	X	X	X	X	X	X	X
Lipase & Coagulation 2: C-protein, S-protein, Anti-Thrombin III	X											
Coagulation 1 : INR, aPTT	X	X+	X	X+	X	X	X	X	X	X	X	X
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen	X	X+		X+								
Coagulation 3 : D-Dimers, optional local TEG		X+		X+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	Xa		Xa		X		X				
TEG, TG	X	X*	X	X*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	Xa	X	Xa	X	X	X	X				X
Chest X-Ray	©											X
ECG	©											
Cardiac US Doppler	©	≠										X
Blood culture or other fluid culture	c											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶ cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X										
Cohort 2a & b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2b only								
Cohort 2c & d : Infusion of 1.0.10⁶ cells /kg body weight with a maximum of 100.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2d only								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a: On infusion day: before infusion

b: On infusion day: before, during and after infusion

%: On infusion day: all parameters are measured prior infusion/platelets measurement to be performed prior and post infusion at 4h, 24h, 48h & 72h

C: Only if performed during the same admission

+: On infusion day: prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion

&: On infusion day: prior and 24h after infusion

©: if not already performed during same admission. If already performed, results collected

*: On infusion day: prior and 4h after infusion

AESI: Only Adverse Event of Special Interest to be reported

≠: cardiac US to be performed after infusion

1. BACKGROUND AND RATIONALE

1.1. CIRRHOSIS, ACUTE DECOMPENSATION AND ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

Cirrhosis is a progressive chronic liver disease characterized by diffuse fibrosis, severe disruption of the intrahepatic venous flow, portal hypertension and liver failure. The course of cirrhosis is divided into two stages. Compensated cirrhosis defines the period between the onset of cirrhosis and the first major complication. During this period, which is relatively long in most patients (>10 years), symptoms are absent or minor, but liver lesions and portal pressure steadily progress. The term decompensated cirrhosis defines the period following the development of ascites (that is, the accumulation of large amounts of fluid within the peritoneal cavity), variceal haemorrhage and/or hepatic encephalopathy. This period is associated with short-term survival (3–5 years). It is increasingly evident that patients rarely die as a consequence of an end-stage irreversible destruction of the liver. Rather, in most patients, the cause of death is an acute deterioration in their chronic clinical condition promoted by a precipitating event — a syndrome termed acute-on-chronic liver failure (ACLF) (Arroyo et al. 2015).

It is of note that definitions on ACLF may differ worldwide. Given the heterogeneity and the importance of identifying patients four major societies/organisations have provided working definitions (APASL, NACSELD, WGO and EASL-CLIF). The common definition of ACLF is 'a syndrome characterised by acute decompensation of chronic liver disease associated with organ failure(s) and high short-term mortality'. According to the CLIF-ACLF definition developed based on the CANONIC study, ACLF is a recognised syndrome characterised by acute decompensation of cirrhosis associated with the failure of one or more organs and, in the more severe cases, system failure. The organs and systems most likely to fail are the liver, kidney, brain, coagulation, circulation and/or lungs. Patients have a high short term mortality of over 15 % at 28 days (Hernaez R et al, 2017). In the CANONIC study approximately 31% of patients admitted to a hospital for Acute Decompensation (AD) of cirrhosis had ACLF at admission (20%) or developed the syndrome during hospitalisation (11%). The common causes of acute decompensation of liver function included bacterial infections, alcoholic hepatitis, and gastrointestinal hemorrhages, but, in more than 40 % of patients, no precipitating event was identified (Moreau et al. 2013). Among patients with Acute Decompensation (AD), subgroups were identified as being at higher risk of progressing to full blown ACLF and thus at higher mortality risk (Arroyo et al. 2015).
Different grading/scoring systems have been developed in order to better determine prognosis and effectiveness of intervention and care. (Hernaez R et al, 2017).

In daily practice, MELD and Child Pugh scores are still strongly relied on to guide clinical care.

The Model for End-Stage Liver Disease, or MELD, is a scoring system for assessing the severity of chronic liver disease. This score is used by the United Network for Organ Sharing (UNOS) and Eurotransplant for prioritizing allocation of liver transplants. New MELD uses the patient's values for serum bilirubin, serum creatinine, sodium and the international normalized ratio for prothrombin time (INR) to predict survival.

Mortality and MELD score are linearly correlated amongst patients with end-stage liver disease listed for OLT with 3-month mortality estimated to be 4%, 27%, 76%, 83%, and 100% for MELD scores of <10, 10–19, 20–29, 30–39, and 40 or more respectively.

The Child–Pugh score is used in clinical practice to assess the prognosis of chronic liver disease, mainly cirrhosis. It was previously used for prioritizing allocation of liver transplants. The score employs five clinical measures of liver disease: total bilirubin, serum albumin, prothrombin time, ascites and hepatic encephalopathy. Each measure is scored 1–3, with 3 indicating most severe derangement. This leads to three Classes with one year overal survival of 100% for Class A, 81% for class B and 35% for class C. (see 11.6)

ACLF has been defined by the CLIF research consortium into four grades based on retrospectively fitting data on severity linked to mortality score (Moreau et al. 2013) (Table 1-1 and Table 1-2)

- ACLF grade 0 concerns 69.1 % of patients admitted to hospital with acute decompensation. The group is defined as no organ failure, single “non kidney” organ failure (ie, single failure of the liver, coagulation, circulation, or respiration) who had a serum creatinine level < 1.5 mg/dL and no hepatic encephalopathy, or as single cerebral failure with a serum creatinine level < 1.5 mg/dL. These patients have a 28-day and 90-day mortality rate of 4.7% and 14% respectively.
- ACLF grade 1 concerns 15.8 % of patients admitted to hospital with acute decompensation. The group is defined as single kidney failure or single non-kidney organ failure with an organ dysfunction (kidney or brain) and has a 28-day mortality rate of 23 %.
- Patients with ACLF grade 2, defined as two failing organs (10.9 % of patients admitted to hospital with acute decompensation) has an intermediate prognosis (28-day mortality rate of 31%).
- Finally, ACLF grade 3, defined as three or more organ failures (4.4 % of patients admitted to hospital with acute decompensation) has extremely high mortality rates, reaching 75 % after 28 days.

Among patients hospitalised with acute decompensation (AD) (pre ACLF according to the CLIF criteria but ACLF according to other classification systems), an analysis revealed five independent variables including age, serum sodium, white cell count, creatinine and INR as useful for defining a scoring system. The high-risk group (CLIF-C AD score > 60) and intermediate risk group (CLIF-C AD score 46-59) respectively have a 3-month mortality of over 30 % and between 2-30 %. The low risk AD group has a 3-month mortality below 2 % (Arroyo et al. 2015).

ACLF may develop at any time during the course of the disease, from compensated to long-standing decompensated cirrhosis. It usually occurs in young cirrhotic patients aged around 50-60 years.The management of patients with ACLF is still poorly defined. The only treatment currently available is orthopic liver transplantation (Bahirwani et al. 2011). However, this treatment is far from ideal due to the shortage of organs for transplantation.

Cirrhotic patients with acute decompensation can only receive supportive treatments, such as antibiotics in case of infection, lactulose in case of encephalopathy, terlipressin and albumin in case of hepatorenal syndrome. However, at this moment, there are no treatments available to stop the inflammatory cascade often accompanying the acute decompensation.

At present, there is no specific treatment for ACLF that improves survival. The MARS system (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not function of other organs or prognosis (Kribben et al. 2012; Banares et al. 2013). On the other hand, circulatory support with terlipressin and IV albumin infusion,

which improves renal function in patients with ACLF, has failed to improve survival as revealed in the two randomized controlled trials published so far in these patients (Sanyal et al. 2008; Martin-Llahi et al. 2008).

Table 1-1 CLIF organ failure score system (CLIF-OF score)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 , renal replacement therapy
Brain (West-Haven)*	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

*West Haven Criteria for Hepatic Encephalopathy (HE)

Table 1-2 ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

The CANONIC study (Moreau et al. 2013) and other investigations strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum CRP levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. The plasma levels of pro-inflammatory cytokines are increased in patients with ACLF as compared to patients with decompensated cirrhosis without ACLF. As indicated before, in 30% of patients, systemic inflammation occurs in association to a bacterial infection and in 25% to acute alcoholic liver injury. However, in the rest of the patients no clear precipitating event can be identified. The mechanism(s) of systemic inflammation in approximately half of the patients with ACLF therefore is unknown.

Systemic inflammation associated to bacterial infections is due to the release of PAMPs (pathogen-associated molecular patterns) by the bacteria. These molecules interact with specific receptors (i.e. TLRs, NLRs) in the innate immune cells (polymorphonuclear leukocytes, monocytes and endothelial cells) and promote inflammation. In non-cirrhotic patients, the presence of circulating PAMPs is normally related to bacterial infections (Wiest et al. 2014). However, in cirrhosis it may also be caused by translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production of PAMPs related to intestinal bacterial overgrowth, increased permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system (Said-Sadier and Ojcius 2012). Therefore, in some patients with ACLF without bacterial infection, systemic inflammation could also be related to PAMPs.

Systemic inflammation in the absence of bacterial infections is frequent in diseases associated to acute tissue necrosis such as fulminant hepatitis or acute alcoholic hepatitis. In these cases, the necrotic or apoptotic cells release damage associated molecular patterns (DAMPs, i.e. fragments of DNA, cholesterol crystals) that also interact with TLRs and other specific receptors and activate the innate immune cells. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines (Martinon et al. 2002).

Systemic inflammation may cause organ failure through different mechanisms. First, it causes arterial vasodilation and impairment in left ventricular function, organ hypoperfusion, tissue ischemia, and cell dysfunction/necrosis. Second, the extension of systemic inflammation to organs impairs cell function and may cause necrosis and/or apoptosis (Gomez et al. 2014, Jalan et al. 2012, Verbeke et al. 2011).

Therefore, new therapeutic approaches targeting systemic inflammation or immunomodulation are warranted. Various cell-based therapies are in development aiming to promote immunomodulation. For example, Mesenchymal Stem Cells (MSCs) originating from the bone marrow, adipose tissue or other tissues are being evaluated in immune-mediated disorders such as graft-versus-host-disease (GVHD), liver transplant rejection, autoimmune diseases (multiple sclerosis, Crohn's disease, …). Some MSC-based therapies are also evaluated in inflammatory liver disorders.

Conclusion on patient population: Based on this information, Promethera Biosciences proposes that the patient population is defined as cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 µmol/L) and MELD < or = 35.

Patients should have coagulation parameters within the ranges below:

- Fibrinogen ≥ 80 mg/ dL
- Platelets ≥ 40.000/mm3

1.2. RATIONALE SUPPORTING THE USE OF HEPASTEM IN ACLF

1.2.1. Introduction

HepaStem is composed of Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC). HHALPC are mesenchymal progenitor cells derived from human livers, expended *in vitro* and cryopreserved until use. Thus, HepaStem is an allogenic off-the-shelf cell therapy product in development phase.

HHALPC are currently in clinical development for the treatment of inborn errors of liver metabolism. For these indications, the cells are expected to integrate into the liver parenchyma and bring the missing enzyme activity to the recipient. The first safety clinical trial has been completed in 2014 (HEP001 study) and a Phase II study is ongoing (HEP002 study). In parallel to this program, the immunomodulatory properties of HHALPC have been investigated and demonstrated *in vitro*. The results support that HHALPC present mesenchymal characteristics and display immunomodulatory properties. Such properties have been demonstrated at various degrees for Mesenchymal Stem Cells (MSCs) originating from other tissues. Pre-clinical and clinical accumulated evidence supports the promising therapeutic potential of MSCs in various immune mediated diseases. Taken together, all these data and the safety profile of HHALPC generated in the HEP001 study support the therapeutic potential of HHALPC for liver inflammatory disorders. The aim of the HEP101 clinical study is to evaluate the safety and explore the biochemical changes when HHALPC are intravenously injected in ACLF patients.

The pre-clinical data, including *in vitro* data on immunomodulatory properties of HepaStem, as well as the clinical safety data generated in the HEP001 study are summarized below.

1.2.2. Pre-clinical data on liver-derived progenitor cells

Liver-derived progenitor cells (similar cells to HHALPC) were first identified, produced and characterized at the PEDI academic lab of Prof. E. Sokal at Université Catholique de Louvain (UCL) in Belgium (referred as Adult-Derived Human Liver Progenitor/Stem cells (ADHLP/SC) in Najimi *et al.* 2007, Khuu *et al.* 2011 and Khuu *et al.* 2013).

Later, the technology of large-scale cell production was transferred to Promethera Biosciences where clinical batches of HHALPC are produced in GMP-accredited facilities. Overall, a preclinical program was conducted in line with regulatory requirements for Advanced Therapy Medicinal Products (ATMPs).

Toxicology *in-vitro or in-vivo* studies aiming to demonstrate the safety, tolerability and tumorigenicity aspect of HepaStem were conducted. *In vivo* studies were performed in rats and mice. They included one study to assess the safety of the intravenous mode of administration. Two studies specifically assessed the risk of tumor formation as this risk has been reported with some stem cell therapies. However, HHALPC are progenitor cells presenting signs of pre-differentiation and no evidence of tumorogenicity was observed in pre-clinical studies: 1) when expanded *in vitro* until cell death, cells entered into senescence; 2) no tumor formation was observed for up to 90 days or 24 weeks post-administration in immunodeficient mice. An *in vitro* study the pro-coagulant activity of HepaStem was confirmed (Please refer to the IB for more details).

In addition, *invitro* studies show that HepaStem cells express variable immunomodulatory surface markers of interest and have immunomodulatory functional effects: HepaStem inhibits the proliferation of activated T-lymphocytes and blocks the maturation of monocytes (see Section 1.2.6). Furthermore, 6 *in-vivo* studies were conducted with HepaStem evaluating the immunomodulatory properties using the IV route of administration and mainly doses of 12.5 10^6 cells/kg. No safety signal was detected based on these *in vivo* studies.

1.2.3. Clinical safety data of liver-derived progenitor cells in genetic diseases

The HEP001 study aimed to evaluate the safety and preliminary efficacy of HepaStem in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome up to 1 year post-treatment.

Method: This partially-randomized, multicenter, open-label, dose-escalation Phase I/II HEP001 study included 20 pediatric UCD or CN patients aged from 6 weeks to 17 years. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg. Three doses were investigated: low (12.5 x 10^6 cells/Kg), intermediate (50 x 10^6 cells/Kg), and high (200 x 10^6 cells/Kg) (4 x 10^9 maximum total cell count). Dose escalation from lowest to highest dose was applied to the first 3 patients from each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in cohorts 1 and 2. HepaStem was infused *via* a percutaneous transhepatic portal catheter inserted under general anesthesia by direct transhepatic puncture under ultrasound or radiologic guidance. Infusions of max. 50 or 100 mL (250 or 500 x 10^6 cells) of HepaStem had to be administered at a 0.5-2 mL/min flow rate, with 2-6 hours or a night in-between. HepaStem infusions were performed under moderate bivalirudin anticoagulation (Angiox®) to prevent coagulation cascade activation. Immunosuppression included Basiliximab (Simulect®) at the time of infusion and tacrolimus (Prograf® or Modigraf®) during the whole study. Methylprednisolone (Solumedrol®) was given on each infusion day as a prophylactic measure to prevent allergic or inflammatory reactions.

Results: 14 UCD and 6 CN patients were included, with age varying from 6 weeks to 17 years and weight varying from 3.4 up to 69 kg. Four patients were assigned to low dose, 5 to medium dose, and 10 to high dose. The high dose could not be fully administered to 5 patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1). In this later case, infusions were stopped as a precautionary measure, no thrombotic event was detected. In practice, total dose administered varied from 23 ml up to 836 ml (115 to 4 180x10^6 cells) given in 1 to 10 infusions spread over 1 to consecutive 4 days. Dose per infusion varied between 23 and 148 mL (115 to 740x10^6 cells), dose per day varied between 23 mL and 402 mL (115 to 2 010x10^6 cells; 3 patients received about 1 750x10^6 cells/day).

Safety: *During hospitalization for HepaStem administration and the following post-infusion days*, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated. SAEs related to HepaStem administration or catheter placement occurred in seven patients. Symptomatic metabolic decompensation occurred in five UCD patients (one between infusions and four at Days 2-4 post-infusion), involving three female adolescent OTCDs, one 10-year-old ASLD, and one 7-year-old ARGD who also exhibited transient transaminase increases, all events resolving within a few days. None did

undergo specific intensified preventive treatment during infusion (i.e. intravenous arginine and nitrogen scavengers, transiently reduced-protein diet), whereas those who did displayed no metabolic decompensation. Of the three cited OTCD adolescent female patients, one developed left portal vein occlusion, after receiving the highest number of cells per day (2 billion over 1 day and 3.5 billion over 2 days). Another UCD patient, an adolescent male OTCD, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removal of the transhepatic catheter that had remained in place for five days (for administering the high dose of 4 billion cells). The cathether used was a curved cathether. Both patients were treated using low-molecular-weight heparin. The first occlusion was not resolved at Month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second fully resolved within 1 month. One CN patient exhibited a transfusion-like reaction treated using methylprednisolone. A week later, infusions were restarted and a similar event occurred, which resolved after stopping infusion. *Beyond the infusion period,* the AEs were in line with those commonly observed in relation with age and morbidity of the studied population. Two patients exhibited donor-specific anti-HLA class I antibodies at Month 6, having disappeared at Month 12 in one patient.

Conclusion: The safety profile was in line with expectations for this new cell therapy, as well as for the infusion procedure, concomitant medications, age and underlying diseases. The safety data support the tolerability of HepaStem, including the infusion process as long as precautionary treatment measures are in place, ie, giving prophylactic urgency regimen to UCD patients and limiting the amount of cells given per infusion and per day. These data laid the ground for further studies in homogeneous UCD or CN patient cohorts or for studies in other indications. (Please refer to the IB for more details).

Based on literature data and Promethera experiences, it can be concluded that liver-derived MSCs, including HepaStem have a pro-coagulant activity. This pro-coagulant activity is also expressed by other MSCs. The pro-coagulant activity might be linked to tissue factor expression, an activator of the coagulation cascade. The procoagulant effect could be modulated by the concomitant administration of bivaluridin during HepaStem infusion in UCD clinical trials in order to prevent, mainly, anticipated thrombotic events. Very high cell doses have been administered intra-portally in the UCD studies in which thrombotic events only occurred at high doses (range: 115 million to 4,1 billion total cells were administered in the portal vein as a split dose in 1 to 10 infusions spread over 1 to 4 consecutive days). Bivaluridin will not be used in the ACLF clinical study as its use has not been validated for late stage cirrhotic patients. Contrary to patients with urea-cycle disorders, coagulation disturbances are common in the late stage chronic cirrhosis population and are linked to liver insufficiency. (see 1.2.5)

1.2.4. Biodistribution of liver-derived progenitor cells injected by peripheral IV route

In a first-in man cohort, conducted in a patient suffering from a severe form of Haemophilia A at the Saint-Luc University Hospital in Brussels, Belgium, by Prof. E. Sokal (2014-2015). The patient received 5 infusions of liver-derived progenitor cells (ADHLSC, similar cells to HHALPC) infused through a peripheral vein route.

In a first step, 25 x 10^6 cells were infused on day 1. The patient tolerated this cell infusion well, without changes in heart rate, oxygen blood saturation or blood pressure. A second infusion of 125 x 10^6 cells was performed on day 2, which was also well tolerated. In the following weeks, infusions of 250 x 10^6 cells

repeated about 1 month apart were also well tolerated. Pulmonary respiratory function tests performed 15 days after each infusion did not show any change as compared to baseline.

In order to follow cell biodistribution, the cells administered on day 1 were labelled with the radiotracer ^{111}Indium. A total body scintigraphy scan was performed 1h, 1 day, 2 days, 3 days and 6 days post-infusion. Results showed that cells were transiently trapped in the lungs and subsequently distributed in the liver, to a lesser extent in the spleen and bone marrow, and specifically in the patient's right ankle joint affected by haemophilic arthropathy. After several days, the cells were mostly found in the liver, spleen, right ankle, and spine, and had disappeared from the lungs. This is in line with the bio-distribution of another type of MSCs administrated in patients (BM-derived MSCs) that demonstrate a similar bio-distribution, with a first pass through the lung; within 24 hours, cells are mainly found in liver, spleen, kidneys and other inflamed areas, by 48 hours, more pronounced presence in the liver is observed. (NDS dossier remestemcel-L, Health Canada).

1.2.5. Immunomodulatory effects of MSCs derived from various tissues

Mesenchymal stem cells (MSCs) have been isolated from multiple tissues such as bone marrow (BM), adipose tissue, Wharton's jelly of the umbilical cord (UC) and placenta. MSCs show *in vitro*: (a) broad potential of differentiation that may be used for tissue repair, (b) secretion of trophic factors that may favor tissue remodeling, and (c) immunoregulatory properties that may restore immunological homeostasis. MSCs were initially tested in clinical setting for tissue repair (Patel 2013 et al. review), i.e. in bone and cartillage disorders (Horwitz et al. 2002) or ischemic heart diseases (Fischer et al. 2013, review). However, the current understanding is that MSCs effects are primarily due to secretion of trophic factors and immunoregulatory properties.

Multiple *in vitro* studies have demonstrated that MSCs affect immune responses through their interactions with cells of the innate and adaptive immune system (T- and B-lymphocytes, natural killer cells, monocytes and dendritic cells). Such effects can be induced by cellular contact and/or secretion of diverse factors or microparticules. Many studies demonstrated inhibitory effects of MSCs on T-cell activation, proliferation, differentiation and effector functions. Involved secreted factors and surface mediators identified include indoleamine-2.3-dioxygenase (IDO), transforming growth factor beta 1 (TGFβ1), hepatocyte growth factor (HGF), IL-10, prostaglandine E2 (PGE2), HLA-G, programmed death-ligand 1 (PD-L1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) among others. Studies have also shown that MSCs can affect monocyte and dendritic cells (CDs) recruitment, differentiation, maturation, and function. For instance, MSCs can inhibit differentiation of monocytes into immature DC as well as maturation of CD and favor generation of regulatory DCs. MSCs can also decrease macrophage polarization toward M1 (pro-inflammatory) while favoring M2 polarization (immunosuppressive with increased IL-10 secretion and phagocytosis). Involved factors identified include IDO, PGE2, IL-10, IL-6 and granulocyte-macrophage colony-stimulation factor (GM-CSF) among others (Ankrum et al. 2014, Glenn et al. 2014, Castro-Marreza at al. 2015, Liu et al. 2014).

Numerous animal studies have tested the efficacy of MSCs in inflammatory mediated diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes Alzheimer's disease, Parkinson's disease, and cardiovascular diseases (Berardis et al. 2015).

The first clinical evaluation of MSCs owing to their immunomodulatory properties was done with allogenic BM-MSCs in graft-versus-host disease (GvHD) (Le Blanc et al. 2008). A company developed a cell product in this indication, currently approved in a few countries (Prochymal® by Mesoblast) (Kurtzberg et al. 2014). As of 2014, about 350 clinical trials, mainly phase 1 or 2, had been registered as evaluating autologous or allogenic MSCs (Ankrum et al. 2014). To date, over 400 clinical trials have been registered in areas emcompassing cardiovascular diseases, osteoarthritis, liver disorders, GVHD, spinal cord injury, kidney failure, skin diseases, muscular dystrophy, aplastic anemia, Crohn's disease, ulcerative colitis, Alzheimer's disease and Parkinson's disease. Available results support that MSCs are promising for the treatment of inflammatory mediated diseases (Vandermeulen et al. 2014, Sharma et al. 2014).

MSCs are also evaluated in liver disorders such as fibrosis, cirrhosis, viral hepatitis and even ACLF. For example, studies have been conducted to assess the efficacy of UC- and BM-MSCs in liver fibrosis. The duration of the follow-up period in these studies ranged from 12 weeks to 192 weeks. At short-term, results supported improvements in one or more of the following parameters: liver function, MELD score, Child Pugh score, ascites score, histology or survival rate (Berardis et al. 2015). An open-label, placebo-controlled trial evaluating the safety and efficacy of UC-MSCs in 43 patients with ACLF associated with hepatitis B virus (HBV) infection showed encouraging results (Shi et al. 2012).

In conclusion, preliminary evidence shows promise for the use of MSCs in liver diseases, nevertheless results from large randomized controlled trials are still needed.

It is clear from consultation of literature publications on cell therapy administration in advanced liver disease including decompensated liver cirrhosis and ACLF that doses of cell therapy protocols tended to be lower as compared to the normal range administered to patients in other immune-modulatory or anti-inflammatory protocols.

The doses and regimens administered to treat patients with chronic liver diseases, range from 0.03 over 0.5 to 1 million MSC cells/kg bodyweight. Different regimens were applied with repeated dosing up to 3 times for the lowest doses (0.03; 0,05 and 0,5 million cells/kg BW repeated 3 times). Most protocols administered the cell infusion intravenously although also other routes of administration were investigated such as intra-splenic, hepatic artery, intrahepatic, intra-lesional route of administration or central venous catheter into the femoral vein. (Berardis et al. 2015)

Based on the literature review, MSC administration is considered to be safe due to the lack of reports of significant adverse effects in the above studies, although a marked heterogeneity was observed among studies with regard to injection dose, frequency of injection, cell source, delivery route and study design. Most of these early studies reported improvements in liver function, ascites and encephalopathy.

In the first cohort of 3 patients in the HEP001 study the lowest dose (12.5×10^6 cells/kg) of the range of doses administered safely in previous studies of HepaStem in urea cycle disorder (UCD) and Crigler Najar pediatric patients was used to determine the dose in the HEP101 protocol. The (low) dose proposed (250

million cells/ infusion; ie. 3.5 10^6 cells/kg BW/infusion) was a reduction of 4x of the lowest dose tested previously (in the HEP001 protocol) and it was thought that it could be safely administered in cirrhotic patients. Additionally, the number of cells administered per infusion would be limited, similar to MSC doses given in immune mediated inflammatory diseases. In retrospect, it was clear that adaptation to the dose level similar to other MSCs given in immune-mediated inflammatory diseases was inadequate and did not take the specific case of severely ill chronic cirrhotic patients with acute decompensation into account.

Therefore, it seems that using a careful approach starting with doses commonly used in reported studies of decompensated cirrhosis and ACLF patients and published as being safe, appears to be an acceptable approach. Also, dose escalation to a maximum of 1.0 million cells/kg BW per infusion should be feasible based on a repetitive dosing schedule. In case of repetitive dosing, the doses will be given weekly, which allows time in case of fibrinolysis for the parameters to be corrected and return to normal.

Pre-clinical immunomodulatory data of liver-derived progenitor cells

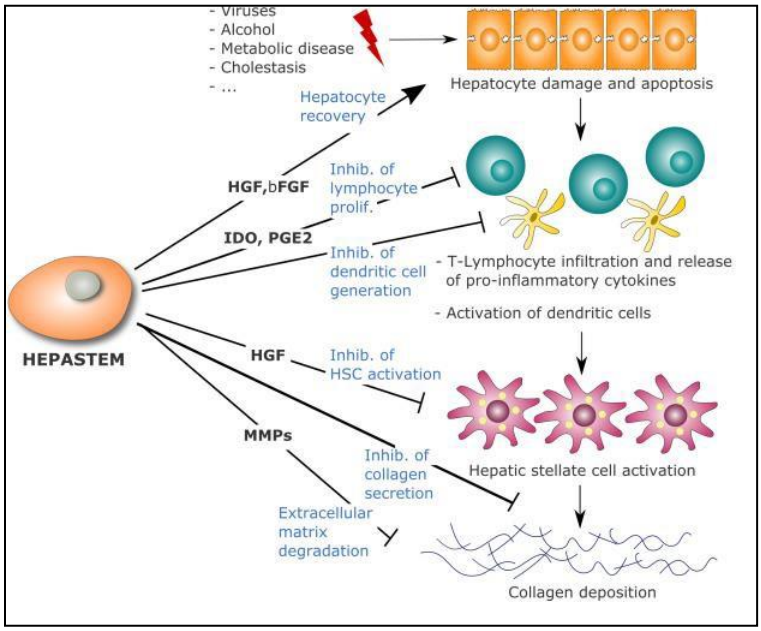
The first transcriptomics and secretomics tests performed on liver-derived progenitor cells (similar cells to HHALPC) grown in the academic lab showed gene expression for a series of pro- and anti-inflamatory cytokines, chemokines and chemokine ligands. Protein expression and secretion was confirmed for pro-inflammatory cytokines, anti-inflammatory cytokines, dual role cytokines, chemokines, and also growth factors (Berardis et al. 2014). Expression of surface markers was evaluated in resting and inflammatory conditions. Several adhesion molecules or surface markers that could have immunomodulatory effects were expressed in both conditions or were increased after inflammatory stimulation (Raicevic et al. 2015). Immunomodulatory effects of these cells could be confirmed *in vitro*. The proliferation of mitogen-activated T-cells was significantly decreased in presence of liver-derived progenitor cells. The level of immunosuppression was comparable to that of bone marrow MSCs (BM-MSCs) (Raicevic et al. 2015). *In vitro* tests also showed the capacity of the cells to modulate the activation of human hepatic stellate cells (HSCs) via a paracrine mechanism. The likely involved mechanisms include (1) inhibition of HSCs proliferation and pro-collagen secretion, (2) up-regulated secretion of anti-fibrotic molecules by HSCs. These effects seem to be mediated at least in part by HGF, highly secreted by these liver-derived progenitor cells (similar cells to HHALPC) (Berardis, PhD Thesis 2014).

Transcriptomics tests performed later on HepaStem produced at Promethera Biosciences confirmed gene expression for a series of pro-inflammatory cytokines (e.g. IL-12), anti-inflammatory cytokines (e.g. TGFb1), dual role cytokines (e.g. IL-6), chemokines, growth factors (e.g. HGF) and also adhesins and other surface markers (e.g. PD-L1, VCAM-1, ICAM-1), and PGES2 enzyme involved in PGE2 synthesis. Secretomics and FACS tests confirmed that HepaStem express a series of immunomodulatory surface markers (e.g. PD-L1) and secreted factors (e.g. HGF, IDO, PGE2, FGF), comparable to those expressed by other human MSCs (data on file, see IB).

In addition, *in vitro* functional tests showed that HepaStem has inhibitory effects on T-lymphocyte and on monocyte activation. In mixed lymphocyte reactions (MLR), the proliferation of T-cells activated by allogenic cells was significantly decreased in presence of HepaStem (data on file, see IB). Monocytes can be differentiated into immature dendritic cells when cultivated with IL-4 and GM-CSF. In presence of HepaStem, cell characterisation supports that this differentiation effect is inhibited. In addition, immature

dendritic cells stimulated with LPS, mimicking an infection, evolve to mature dendritic cells. In presence of HepaStem, measurements of surface markers and secreted factors support that this maturation is inhibitied. These inhibitory effects are observed in case of direct or indirect HepaStem contact, supporting both a cell-cell contact and a paracrine effect (data on file, see IB). The potential effects of HepaStem are summarized in Fig 1-1.

Figure 1-1 Expected Mode of Action of MSCs and HepaStem in inflammatory diseases



Testing immunomodulatory effects in animal models presents important limitations. Indeed, in immunocompetent animals, human cells may be very rapidly eliminated due to the xenogenic reaction. In immunodeficient animals, detection of immunomodulatory effects would be of poor relevance. The development of inflammation and fibrosis is quite limited in such animals.

In conclusion, *in vitro* data on HepaStem, when compared to literature data on MSCs, support the potential of HepaStem to induce immunomodulatory effects *in vivo* in immune mediated diseases.

1.2.6. Expected Mode of Action of HepaStem in ACLF

HepaStem is expected to have a combined systemic and local effect in the liver. Indeed, after IV administration, cells have a main homing to the liver. They are expected to play an immunomodulatory role, by direct cell-to-cell interaction with immune cells of the recipient, and by paracrine effects through the various cytokines, chemokines, MMPs and growth factors they may secrete. For instance, activation of monocytes and Kupffer cells, the liver resident macrophages by PAMPs and DAMPs are thought to play an important role in the exacerbated inflammatory response observed in ACLF. According to *in vitro* data, HepaStem could affect monocyte and dendritic cell (DC) recruitment, differentiation, maturation and function through cell contacts or paracrine effects. All these data support the potential *in vivo* immunoregulatory effect of HepaStem on monocytes in ACLF patients. By these combined effects, it is expected that HepaStem will play a favourable role in restoring the immunological imbalance observed in ACLF, helping to resolve this acute event, meaning improving liver and renal function, systemic

hemodynamics, coagulation parameters and respiratory parameters, and also resolving hepatic encephalopathy. This will be further explored in this and further clinical studies.

1.2.7. Expected Benefits of HepaStem

Proposed mechanisms of action: after intravenous administration of HepaStem, cells are expected to circulate into the blood network where they can exert a systemic immunomodulatory action. At the same time, they have a main homing into the liver where they can thus also exert some important local immunomodulatory effects. They are expected to play their immunomodulatory roles through direct cell-to-cell interaction and through paracrine effects via the various cytokines, chemokines, MMPs and growth factors they may secrete. HepaStem could affect monocytes and DC recruitment, differentiation, maturation and function through cell contacts or paracrine signalling. HepaStem could also alter the proliferation and activation of T-lymphocytes that are another dysregulated cell type of the immune system in ACLF. In addition to modulate the behaviour of immune cells, HepaStem could modulate the proliferation and activation of hepatocytes and hepatic stellate cells and thus their secretory profiles, helping in this way the liver function recovery. The current *in vitro* and *in vivo* data, based on the scientific literature, and sponsor *in vitro* results, support all these potential immunomodulatory effects of HepaStem in ACLF patients.

Proposed clinical significant benefit: by these combined effects, HepaStem could play a favourable role in restoring an immunological balance in ACLF patients or patients at risk of ACLF, improving organ failure scores, improving clinical status, possibly leading to a resolution of this acute event and demonstrating improvement of transplantation free survival.

Considering the unmet medical need: i. the emergency to treat cirrhotic patients with Acute Decompensation (pre-ACLF or ACLF) due to the high mortality rate; ii. the shortage of healthy donors and the need of livers in the context of liver transplantation; iii. Concerns raised recently regarding artificial liver support; and iv. the mechanism of action of HepaStem, we can say that all these factors are in favour of a promising favourable benefit/risk balance for HepaStem. The exact profile of which patients will benefit most is under investigation, and also subject of this safety study.

1.2.8. Justification of study design, population selection, dose regimens and mode of administration

The study is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts with approximately 21 hospitalized patients in total. The study will include patients with an acute decompensation of cirrhosis and with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 35, excluding patients with circulatory, respiratory failure or severe coagulations disorders. It is planned to have a first group of 6 patients approximately (cohort 1) being administered with the low dose.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days.

On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone or equivalent was given 15 to 30 min before each HepaStem infusion.

2 patients experienced an episode of severe bleeding. Therefore, it has been decided to reduce the dose in the low dose cohort to $0.25.10^6$ cells/kg bodyweight with a maximum of 25.10^6 cells in a single infusion. A reduction of minimum 10 times the dose previously used.

For cohort 1b: Approximately 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg bodyweight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion

Once this has been proven safe, a second group of approximately 3 patients (cohort 2a) will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: Approximately 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg bodyweight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation)

Additionnally, approximately 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort.

For cohort 2c: Approximately 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion.

For cohort 2d: Approximately 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).

HepaStem will be administered by a peripheral or a central vein. The primary objective is to evaluate safety of HepaStem at Day 28 of the active study period.

Study design

In the present study, HepaStem will be administered for the first time through central or peripheral IV route to cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, and escalating to a higher dosage in a second cohort of 12 patients, appears to be an acceptable approach in patients with ACLF or with acute decompensation at risk of developing ACLF for whom no specific therapeutic or curative treatment exist. (See section 1.1)

HepaStem administration will be started rapidly after hospitalisation and will be completed within 1 day (cohort 1b, 2a, 2c) or within 1 week (cohort 2b, 2d). This period is expected to correspond approximately to the usual (minimal) hospitalisation stay of patients with or at risk of developing ACLF. As ACLF and/or Acute Decompensation of cirrhosis is an acute event, rapidly evolving, with a high mortality rate within the first weeks (Moreau et al. 2013), a rapid treatment seems a key element to avoid further organ dysfunction or failure. HepaStem is intended to provide improvement in the short term by its immunomodulatory and anti-inflammatory properties.

The main objective of the study is fixed at Day 28 of the active study period in order to evaluate the safety of the infusion. Beyond this period, other intercurrent events may represent confounding factors for the evaluation of Hepastem effects, such as stopping abstinence from alcohol. Nevertheless, after this 28-day active period, patients will be followed-up up to 1 year post HepaStem administration initiation.

Secondary objectives also include exploration of efficacy parameters in ACLF patients. Collected data on parameters used for evaluating ACLF and liver disease evolution will serve as a basis for selecting efficacy parameters and defining the design of future efficacy clinical studies.

Study population

The patient population is defined by cirrhotic patients with acute decompensation (may present with ascites, increase of bilirubin, with or without encephalopathy) with a serum total bilirubin of ≥ 6 mg/dL (≥100 umol/L) and MELD < or = 35 (see section 1.1).

Patients should have coagulation parameters within the ranges below:

- Fibrinogen ≥ 80 mg/ dL
- Platelets ≥ 40.000/mm3

Dose

Administration of 250 millions cells (50 mL of HepaStem) per infusion, with 4 infusions given over 2 weeks – as planned for the first cohort 1a- corresponds approximately to 3.5 millon cells/kg/infusion and a total dose of 14 million cells/kg (assuming a patient weight of 70 kg). Administration of 500 millions cells (100

mL) per infusion, with 4 infusions given over 2 weeks – as planned for the second cohort - corresponds approximately to 7 millon cells/kg/infusion and a total dose of 28 million cells/kg (assuming a patient weight of 70 kg). The first selected dose (250 million cells per infusion) is close to MSC doses given in other trials for immune-mediated inflammatory diseases (Section 1.2.5), therefore, it was expected to show a similar safety and efficacy profile. It corresponded also to the high dose of liver-dirived progenitor cells (similar cells to HHALPC) administered via IV to the heamophila patient (see 1.2.4).

Due to the severe bleeding that occurred in 2 of the 3 patients that received 250 10^6 cells (50 mL of HepaStem) per infusion, the next selected dose (low dose cohort 1b) will be reduced to $0.25.10^6$cells/kg bodyweight (with a maximum of 25.10^6 cells per infusion) administered in a single infusion (at least a 10x reduction of the dose administered in cohort 1a).

The second selected dose represents a two-fold increase from the dose in cohort 1b ($0.5.10^6$cells/kg bodyweight (with a maximum of 50.10^6 cells per infusion) still in the range of doses reported for MSCs and more in the range of doses administered in the specific case of severely ill chronic liver disease patients with ACLF and acute decompensation of cirrhosis. (see1.2.5)). (for additional information, please refer to the rationale for changes).

The third and fourth selected dose represents a two-fold increase from the cohort 2a and 2b. These dose still in the range of doses reported for MSCs and more in the range of doses administered in the specific case of severely ill chronic liver disease patients with ACLF and acute decompensation of cirrhosis. (see1.2.5)). (for additional information, please refer to the rationale for changes dated 28June2018).

Mode of administration

HepaStem will be administered in a peripheral or a central vein without concomittant anti-coagulation medication. In the HEP001 study, HepaStem was administered directly via the portal vein under an anti-coagulation with bivalirudin (in addition Hepastem formulation contains heparin at 10 IU/ml).

When infused by peripheral IV route, based on a biodistribution test in a patient with normal liver, cells were shown to have, as expected, a transient passage in the lungs, without inducing neither lung damage nor any respiratory symptoms, before homing mainly to the liver. This cell biodistribution is relevant for ACLF indication as the main expected mode of action of HepaStem is based on cell interaction with immune cells and on paracrine effects in the liver, but systemic effects may also be useful. On the other hand, ACLF patients present portal hypertension and disturbed portal vein flow, contraindicating portal vein access. Peripheral or central IV route is therefore justified in cirrhotic patients with ACLF or with acute decompensation at risk of developing ACLF, it also can allow repeated infusions over time. This mode of administration is expected to improve applicability and acceptability of the treatment.

HepaStem has a known procoagulant activity due to high expression of Tissue Factor and low expression of Tissue Factor Pathway Inhibitor (Section 1.2.2). In this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication (bivalirudin). Indeed, the risk of thrombosis is thought to be lower with this route compared to portal vein route used in the HEP001 study as the blood flow in a medium or central vein is expected to play a diluting effect. In addition, the number of cells administered per infusion will be limited, similar to MSC doses given in immune mediated

inflammatory diseases (Section 1.2.5). *In vitro* tests showed that BM-MSCs also have a procoagulant activity comparable to liver-derived progenitor cells (similar cells to HHALPC) (Stephenne et al. 2012), nevertheless literature reports show that MSCs were safely administered by IV route without anticoagulation medication, even if triggering activation of the clotting system (Moll et al. 2012, Moll et al. 2015).

However, liver failure results in a state of "rebalanced hemostasis" marked by a decrease in both pro-coagulation and anticoagulation factors. Patients with severe liver disease are not auto-anticoagulated. In essence, patients with severe liver disease, acute and/or chronic, have a tenuous rebalanced hemostasis that is easily perturbed by various disease states and concomitant medications and invasive procedures. Bleeding events including severe forms are common in these end-stage liver disease patients. The events of epistaxis and bleeding from puncture sites that occurred in 2 patients in cohort 1a (in retrospect a high dose in late stage cirrhotic patients), have been recognised in the literature as case reports. It was also stated that epistaxis as an overlooked cause of massive haematemesis in cirrhosis should be added to the list of upper GI bleeding (Johal et al 2003). Hence, cirrhotic patients including ACLF patients are at increased risk of bleeding or thrombosis. Therefore, dose reduction from normal ranges applied in other immune-modulatory diseases, modification of inclusion criteria and increased surveillance of liver and coagualation parameters is indicated.

In this study, no immunosuppression will be co-administered with HepaStem. An immunosuppression treatment was given in the HEP001 study in order to prevent a potential cell rejection. An immunosuppressive treatment would be contraindicated in ACLF patients presenting an immunological imbalance and being at high risk of severe infections. Furthermore, in ACLF, the expected mode of action of HepaStem is based on immunomodulation rather than on long-term cell engraftment.

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone or equivalent will be given 15 to 30 minutes before each HepaStem infusion (as in Lublin et al. 2014).

2. OBJECTIVES OF THE STUDY

2.1. PRIMARY OBJECTIVE

To assess the safety of different regimens of HepaStem in cirrhotic patients presenting with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

2.2. SECONDARY OBJECTIVES

Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28, up to Month 3 and up to Year 1 post first HepaStem infusion.

Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3. INVESTIGATIONAL PLAN

3.1. GLOBAL STUDY DESIGN

This is an interventional, multicenter, open, non-controlled study of different dose regimens of HepaStem given in subsequent cohorts with 18 (up to 21) hospitalized patients in total.

5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next patients enrolled will complete each dose cohort in a step wise approach and will receive a lower dose following SAEs observed in patient 2 and patient 3 in the cohort 1a with high dose of cells).

Approximately three patients will be enrolled in cohort 1b.

Approximately fifteenother patients will be enrolled in cohort 2 (cohort 2a + 2b + 2c + 2d)

Approximately 3 patients of the cohort 2 (cohort 2a) will receive twice the dose compared to the cohort 1b.

Approximately 3 patients of the cohort 2b will receive up to 2 times the dose given to cohort 2a one week apart.

Additionally, approximately 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort.

The patients of the cohort 2c and 2d (approximately 3 patients for each subcohort) will receive up to 2 times the dose given in to the cohort 2b.

The study will recruit patients who are hospitalized for Acute Decompensation of cirrhosis and/or who develop ACLF during hospitalisation.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

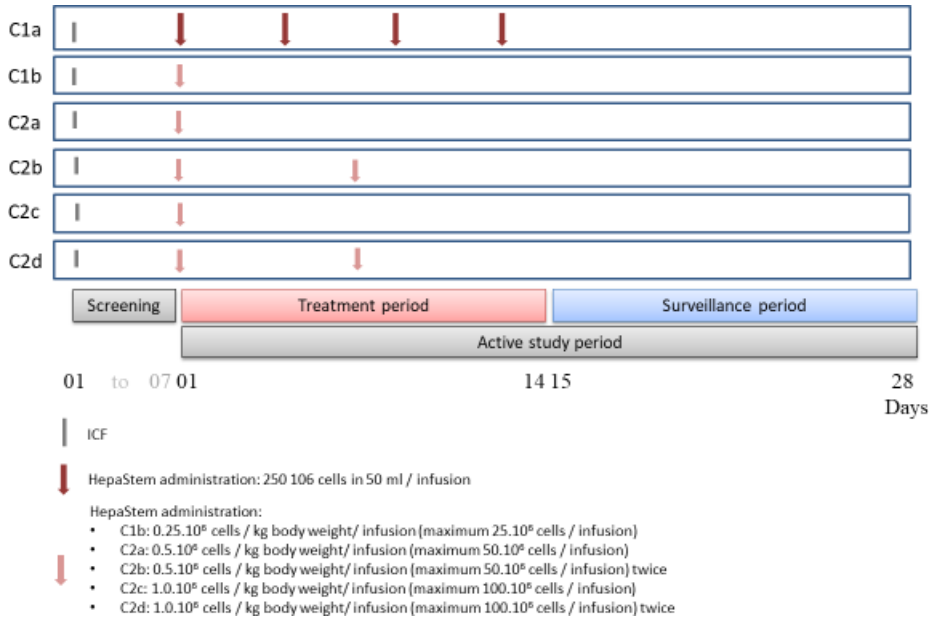
Screening period: Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days). The duration of the screening period plus the active period will last up to 35 days (± 2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Two dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 3-1 and described in Section 5.2.

Figure 3-1 Study scheme of active study period



Planned schedule:

For cohort 1a, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion must be respected between infusion days.

In cohort 1a, 250 million cells in 50 ml were administrated on each infusion day, leading to a total of 1 billion cells if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted

HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before each HepaStem infusion.

Actual schedule:

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone or equivalent was given 15 to 30 min before each HepaStem infusion.

Planned schedule:

For cohort 1b: Approximately 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion.

For cohort 2a: Approximately 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: Approximately 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).

Additionally, approximately 3 patients considered as more severe ACLF patients (INR >2) will be included in the cohort considered as safe by the Safety Monitoring Committee (2a or 2b) for less severe patients (INR <2) which will be the cohort completed at the time of approval of the related amendment before to start inclusion in the next dose cohort.

For cohort 2c: Approximately 3 patients will receive HepaStem in a single infusion ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells). One infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before HepaStem infusion.

For cohort 2d: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($1.0.10^6$ cells per kg bodyweight with a maximum of 100.10^6 cells per infusion). 2 infusions of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see 4.4 - Criteria for study treatment discontinuation).

Patients will be treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of members, all external and independent to Promethera Biosciences. (See Section 9.13):

In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

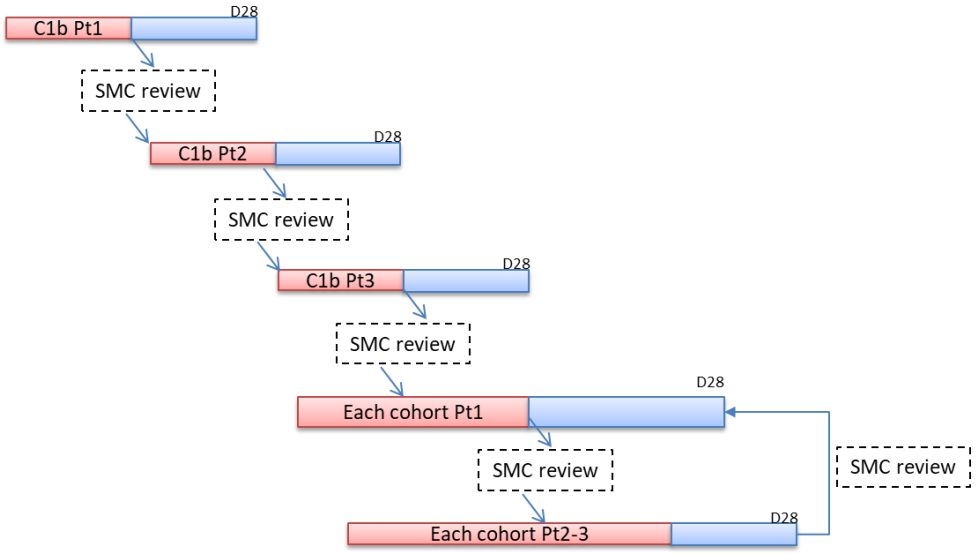
As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a, 2b, 2c and 2d), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- For the first cohort (1b), when the second evaluable patient has received HepaStem infusion (complete scheme or premature stop), the patient data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

More specifically, based on the patients' parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a single administration of $0.5.10^6$ cells/kg body weight).

Figure 3-2 Stepwise inclusion approach under supervision of Safety Monitoring committee



The study assessments are described in Section 6.

Furthermore, the 3 first patients of the cohort 2a,2b, 2c and 2d will be treated according to a sequential approach. For these patients, the first infusion will be performed only after the previous patient has completed the treatment period (with complete scheme or premature stop). The sequential approach will be under the control of Promethera (based on review of eligibility criteria by the medical monitor and HepaStem delivery). In case of safety signal, the SMC will be involved in the AEs review and evaluation, and the SMC will advise on further inclusion.

These measures (SMC meetings and sequential treatment for the 3 first patients in each cohort) will allow respecting the progress of dose levels with limited risk for the patients.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the safety follow-up period.

After completion of this study, patients will be invited to be followed-up in the Patient Long-term follow-up registry for 5 years.

3.2. STUDY ENDPOINTS

3.2.1. Primary endpoint: Safety

- AEs reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to IMP and/or IMP administration procedure

  The AEs will include but will not be limited to clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28.

  The relationship will be assessed based on investigator assessment and in addition by the SMC and the sponsor's pharmacovigilance in line with the ATMP guideline.

3.2.2. Secondary Endpoints:

- Clinical efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Mortality
  - Liver transplantation
  - Disease scoring: CLIF-OF score, CLIF-C ACLF score, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh score.
- Biological efficacy parameters will be assessed at Day 28, Month 3 and Year 1
  - Bilirubin, creatinine, INR and albumin values

3.2.3. Long term safety follow-up

- Adverse Events of Special Interest will be summarized at Month 3 and Year 1
  - SAE with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, transplantation and outcome of the transplantation
- New ACLF episode will be sumamrized at Month 3, Year 1

3.3. RANDOMIZATION

This study is not randomized but sequential, occurring in successive cohorts of patients.

3.4. JUSTIFICATION OF STUDY DESIGN AND DOSE

The justification is provided in section 1.2.8.

3.5. STUDY CENTERS

The study is planned to be conducted in up to 25 centers in Europe.

3.6. STUDY DURATION

The enrolment period will last approximately 12 months. Each evaluable patient will participate for up to five weeks (35 days ($\pm$ 2 days) in the screening plus active treatment period), and therafter for an additional period up to 1 year post first HepaStem infusion (D1)n the safety follow-up.

4. STUDY POPULATION SELECTION

4.1. STUDY POPULATION

Patients will be recruited at hospitals with specialized hepatology and intensive care units. The hospitalisation unit will be adapted according to the medical status of the patient and the organization of study center hospital. Patients with a low CLIF-OF score will be more likely included in the hepatology department (standard or intermediate care unit), while the patient with high CLIF-OF score will more likely be included in the Intensive Care Unit.

Patients will remain hospitalised at least during the treatment period. During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Cirrhotic patients with Acute Decompensation at risk of developing ACLF at first evaluation post-admission and/or developing ACLF during hospitalisation will be assessed for inclusion. After obtaining informed consent, the screening period may last maximum 7 days, time to complete the screening process and to deliver HepaStem to the center. This period will include confirmation of eligibility criteria.

4.2. INCLUSION CRITERIA

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 years old.
2. Informed Consent.
   N.B: In case of hepatic encephalopathy, if the patient is not able to understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.
3. Cirrhosis as diagnosed by
   - liver histology or
   - clinical and imaging examination (may include fibroscan).
4. Patient with Acute Decompensation of cirrhosis
5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)

4.3. EXCLUSION CRITERIA

Any of the following criteria will exclude a patient from the study:

1. Thrombosis of the portal vein.
2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.

   Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.
3. Ongoing uncontrolled bleeding.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
5. Clinical evidence of Aspergilus infection.
6. Circulatory failure defined by inability to maintain a mean Blood pressure ≥ 70 despite use of vasopressors.
7. Mechanical ventilation due to respiratory failure.
8. Coagulation disorders defined as :
   - Fibrinogen < 80 mg/dL
   - Platelets < 40.000/mm^3
9. Major invasive procedure within the week before the infusion (including but not limited to tranjugular liver biopsy)
10. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.
11. MELD score > 35.
12. Previous organ transplantation and/or ongoing immunosuppressive treatments.
13. Postoperative-decompensation following hepatectomy.
14. Renal failure due to chronic kidney disease.
15. Clinically significant left-right cardiac shunt.
16. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
17. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. In case of suspicion of HCC, all exam should be done to confirm or not the diagnosis prior enrolment.
18. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
19. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
20. Participation to any other interventional study within the last 4 weeks.
21. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

4.4. CRITERIA FOR STUDY TREATMENT DISCONTINUATION

Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration for a patient are the following:

- **Transitory discontinuation:** Coagulation disorders considered as significant (Fibrinogen < 80 mg/dL, or Platelets < 40.000/mm^3) by the PI prior to each infusion should preclude the administration of Hepastem.
- Thrombosis of the portal vein should preclude the administration of Hepastem.
- Tranfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.

Infusions may be restarted after recovery. If planned infusions are not performed within treatment period ($\pm$ 2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock, severe worsening of hepatic function.

The reason of study treatment discontinuation will be documented, and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

4.5. STUDY WITHDRAWAL CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

The reason for withdrawal of the Patient will be documented. If possible, blood samples and study data will be obtained to complete the pertinent tests and data collection at the time of patient withdrawal. No patient can be re-enrolled into the study after having been definitively withdrawn from the study.

4.6. SCREENING FAILURE AND PATIENT REPLACEMENT

Enrolled patients who signed the Informed Consent but do not meet the inclusion/exclusion criteria at the end of the screening period (i.e. detection of an exclusion criteria) and will not receive any infusion and will be considered as screening failure. Enrolled patients not receiving any HepaStem infusion will be replaced.

Any Patient receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

5. TREATMENTS OF PATIENTS

5.1. DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

The composition of the Investigational Medicinal Product (IMP) HepaStem is a 5-ml suspension of HHALPC (50 x 10^6 cells/ml) mixed with Cryostor® CS-5. HepaStem is stored in 50 ml-vials at maximum -130°C and reconstituted with 45 ml of HepaStem Diluent at the study center before administration. The concentration of the reconstituted suspension is 5 x 10^6 cells/ml.

5.1.1. Description of the Investigational Medical Product

Livers from donors are enzymatically and mechanically treated. The resulting cell suspension is frozen and stored at maximum – 130°C. Liver cells suspension is the starting material for HepaStem manufacturing. At Promethera Biosciences, liver cell suspension is thawed and washed prior to seeding in cell culture flasks in aseptic conditions. Cells are cultured in appropriate medium to promote liver progenitor cells to emerge. Once liver progenitor cells have proliferated and colonized the growth surface, cells are detached with a recombinant trypsin-like enzyme and plated again. Then, cells are expanded on increasing growth surfaces for additional passages prior to the harvest.

After harvest, cells are washed and concentrated in Phosphate-Buffered Saline then mixed in CryoStor® CS-5 (cryopreservation solution containing 5% dimethyl sulfoxide [DMSO]) to reach a final cell concentration of 50 x 10^6 total cells/ml; 50-ml vials are filled with 5 ml of cell suspension (Drug Product HepaStem) for a final dose of 250 x 10^6 total cells/vial. The HepaStem is then stored in the vapor phase of liquid nitrogen tank at maximum -130°C (Table 5-1).

Table 5-1 Composition of HepaStem (frozen)

	Composition (5 ml)
ACTIVE SUBSTANCE HHALPC	50 x 10^6 cells/ml
EXCIPIENT Cryostor-5% DMSO	To 5 ml

Vials of frozen HepaStem are shipped to the clinical centers in dry-shipper at maximum 150°C where they can be stored for several days. They may also be shipped on dry-ice and stored between -70°C and -90°C.

The exact expiration date will be indicated on each vial. Further instructions are provided in the HepaStem Manual.

5.1.2. Reconstitution of Investigational Medicinal Product

HepaStem is delivered in a 50-ml plastic vial containing 5 ml of frozen cell suspension concentrated at 50 x 10^6 cells/ml equivalent to 250 x 10^6 cells/vial. Prior administration to patient, HepaStem will be reconstituted by healthcare professionals with 45 ml of diluent. HepaStem Diluent is a solution composed of human albumin, heparin sodium and sodium bicarbonate (Table 5-2).

Table 5-2 Composition HepaStem (reconstituted)

	Composition (50 ml)
HHALPC	5 x 10^6 cells/ml
Sodium Bicarbonate	0.076%
Human albumin	4.45%
Heparin	9 IU/ ml

After reconstitution, the residual DMSO concentration has been shown to be below the limit of 5.5 mg/ml, which is the limit approved by the Paediatric Committee of European Medicines Agency (EMA) considering a maximum of 0.44 g DMSO/kg/day.

Please refer to the IB for further information.

Special precautions for disposal and other handling

- The reconstitution of HepaStem should be performed by trained study staff Health Care Professional. The maximum delay between the reconstitution and the end of the infusion is 120 minutes. HepaStem reconstitution is expected to last 5 minutes. Time required from beginning to end of infusion of 50 ml of HepaStem is expected to be about 50 minutes.
- The following equipment is needed to perform HepaStem reconstitution:
  - 1 x 50-ml HepaStem vial with 5 ml of cells frozen in Cryostor® CS-5
  - 1 x 50-ml HepaStem Diluent vial with 47.5 ml of diluent
  - 1 x reconstitution device pack containing sterile mini-spike, sterile 50-ml syringes, sterile Luer lock stopper and 70% isopropyl alcohol (IPA)-saturated prep pads.
- No particular individual protective clothing is required for the reconstitution of HepaStem. Wearing a laboratory coat and safety glasses are recommended. There is no specific air cleanliness or biosafety level requirement for the reconstitution of HepaStem. The reconstitution will be performed on a clean and cleared bench at room temperature (15-25°C).
- The exact dosage (volume) of Hepastem infused to the patient will be calculated based on the weight of the patient on the day of infusion (0.25.10^6 cells per kg bodyweight with a maximum of 25.10^6 cells/infusion (5 mL) for cohort 1b, 0.5.10^6 or 1.0.10^6 cells per kg bodyweight with a maximum of 50.10^6 or 100. 10^6cells/infusion (10 mL) for cohort 2.)
- As the exact volume to infused can be low (depending on the patient's weight), it is recommended to flush after the infusion physiological solution (NaCl 0,9%) to ensure that all the product is infused.

The full procedure is described in the the HepaStem Manual. Briefly, the mini-spike and the 50-ml syringe will be assembled. The diluent vial septum will be pierced with the mini-spike and 45 ml of the diluent will be transferred into the syringe. Then, the HepaStem vial septum will also be pierced with the mini-spike and the totality of the diluent will be transferred into the HepaStem vial. The reconstituted product will be homogenized very gently until the cell suspension is homogeneous. The mini-spike and the syringe will

be disassembled and the reconstituted HepaStem syringe will be immediately transferred to the patient bed inorder to be promptly infused

5.2. IMP DOSAGE AND SCHEDULE OF ADMINISTRATION

After reconstitution, HepaStem will be infused intravenously through a peripheral catheter or through a central line. The choice will be at the discretion of the investigator for each patient and for each study treatment administration, depending on available and possible IV access at the time of infusion.

The infusion rate shall not exceed 1 ml per min. Actual infusion rate will be documented and collected in the eCRF.

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (± 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given.

For cohort 1b: Approximately 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells per infusion). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapated accordingly. A single infusion is expected to last minimum 5 min (for 5 mL of reconstituted Hepastem).

For cohort 2a and 2b: Approximately 3 patients will receive HepaStem in a single infusion (cohort 2a) and approximately 6 patients or in 2 repeated infusions one week apart (cohort 2b). The dosage of Hepastem per infusion will be $0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion).

For cohort 2c and 2d: Approximately 3 patients will receive HepaStem in a single infusion (cohort 2c) or in 2 repeated infusions one week apart (cohort 2d). The dosage of Hepastem per infusion will be $1.0.10^6$ cells per kg body weight with a maximum of 100.10^6 cells per infusion).

Each infusion of maximum 20 ml reconstituted HepaStem suspension will be given. In case the patient's bodyweight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single infusion is expected to last maximum 20 min (for 20 mL of reconstituted Hepastem).

The full procedure describing how to adapat the volume of HepaStem to be administrated to the patient's body weight is in the HepaStem Manual.

5.3. METHOD OF APPLICATION

The patients included in the study can be hospitalised in intermediate, ICUs or standard unitsdepending on the medical status of the patient and the organisation of study center hospital. Regardless of the unit of hospitalization, patients will remain hospitalised at least during the treatment period, with a close monitoring of each patient. During HepaStem infusion, a continuous monitoring of the vital signs of the patient is required.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a

specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Hepatic ultrasonography and Doppler ultrasound of the portal vein should be performed on each treatment day before HepaStem infusion in order to check presence of portal vein flow and arterial flow and to exclude thrombosis of the portal vein (see Section 5.5.1).

Vital signs should be measured prior to, during, and after each HepaStem infusion. Vital signs include body temperature, arterial pressure, heart rate, respiratory rate, and pulse oximetry saturation.

A single bolus of 100 mg hydrocortisone or equivalent will be given 15 to 30 min before first HepaStem infusion of the day.

Before infusion, the homogeneity of the cell suspension has to be checked. During infusion of HepaStem, the syringe should be regularly gently moved in order to avoid cell sedimentation : the syringe has to be inversed 10 times every 3 minutes.

It is very important to ensure a good hydration of the patient before, during and after the cell infusion procedure.

5.4. POTENTIAL COMPLICATIONS RELATED TO HEPASTEM ADMINISTRATION

Based on risks reported in studies with cell therapy or biologics, risks observed with HepaStem administered in the portal vein in pediatric patients (HEP001 study, see Section 1), and risks observed with the infusion of HepaStem in the cohort 1a, main potential risks linked to HepaStem therapy may include:

- at short-term: activation of coagulation cascade due to tissue factor present on cells which might lead to thrombosis or to consumption of coagulation factors and subsequent bleeding; respiratory disorder as cells first transit to the lungs; hypersensitivity reaction or infusion reaction as it may occur with biologic products;
- at mid- or long-term: biodistribution in hepatic and non-hepatic organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of allogenic cells which may eventually lead to cell rejection.

Furthermore, ACLF patients are often in a poor medical condition, with multiple organ dysfunction or failures predisposing to precipitating major adverse reactions including fatal outcome.

Measures to be taken to minimize the risks potentially attributable to HepaStem administration are described here below. Besides the risks mentioned below, there might be other, at this time, unknown risks.

5.4.1. Risk and Benefit assessment

ACLF patients or patients at high risk to develop ALCF are at high mortality risk and there is currently no specific treatment for these patients. Orthotopic liver transplantation is often not a possible option for these patients. By its potential combined effects, HepaStem could play a favourable role in restoring an immunological balance in pre-ACLF / ACLF patients, leading to a resolution of this acute event and showing

improvement of organ function and transplantation free survival. The main identified risks linked to HepaStem are activation of the coagulation cascade and may lead to thrombosis (observed in UCD patients) or bleeding (observed in ACLF patients). The safety measures described below (see section 5.5) are recommended to minimize the risks of the administration of HepaStem in ACLF or pre-ACLF patients at high risk of short term mortality.

5.5. MEASURES TO MINIMIZE RISKS

The Patients will be closely monitored and evaluated daily as inpatients and at planned study visits as outpatients. In addition, the safety of the

Before each infusion, the investigator will have to make sure the patient has the minimum criteria to receive HepaStem (see 4.4 - Criteria for study treatment discontinuation).

Patients will be under supervision of the Safety Monitoring Committee (refer to Section 9.13).

5.5.1. Coagulation disorders and lung disorders

Although HepaStem has a known procoagulant activity due to the presence of tissue factor (see Section 1), in this study, HepaStem will be administered by peripheral or central IV route without anti-coagulation medication. Indeed, the risk of thrombosis is thought to be lower with low doses administered by the IV route compared to high dose administered by the portal vein route as in the HEP001 study. The blood flow in a medium or central vein is expected to play a diluting effect.

Number of cells administered for the cohort 1a, 2a,2b, 2c and 2d per infusion will be maximum 25.10^6 cells (cohort 1a) 50.10^6 cells (cohorts 2a and 2b) or 100.10^6 cells (cohorts 2c and 2d)and the recommended infusion rate is low, at 1 ml/min or 5 millions cells/min. The lower dose regimen will be applied before the higher one. These doses are in the very low range compared to HepaStem doses given to pediatric patients in HEP001 study. They are close to MSC doses given in inflammatory diseases where MSCs were safely administered by IV route without anticoagulation medication and re-adjusted to the range of MSC doses given in decompensated cirrhotic and ACLF patients (See Section 1).

Furthermore, **the coagulation parameters will be closely monitored** prior and after the infusion process at 4h, 8h, 12h, 24h, 48h and 72h post infusion. (Including INR, aPTT, fibrinogen, D-Dimers, coagulation factors (pre and 24h post infusion), and TEG (optional, only if measurement can be done locally and up to investigator's judgment)

The **coagulation status will be monitored** regularly during the active treatment period. In addition, patient will be evaluated at baseline based on thomboelastogram (including EXTEM and FIBTEM tests) and thrombin generation (with thombomodulin). The results will be useful to document the impact of HepaStem infusion on the coagulation cascade.

Patients with recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion, and patient with risk of bleeding (defined by recent major invasive procedure, non controlled gastrointestinal hemorrhage and/or coagulation disorders) will be excluded from the study.

In case major changes in the coagulation parameters and/or clinically significant bleedings suggestive of important coagulation factors consumption occur, according to the investigator's judgement, it could be envisioned to administer coagulation factors in the form of fresh frozen plasma (FFP), fibrinogen concentrate (ie RiaSTAP), and/or antifibrinolytics (ie tranexamic acid). (cfr. both study patients in cohort 1a responded well to treatment with FFP and/or addition of coagulation factors).

In case of clinical signs or suspicion of pulmonary embolism, the investigator should perform exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigraphy.

Cells will be diluted before reaching the pulmonary capillary circulation as lungs are their first organ encounter. Nevertheless, a **close monitoring of the respiratory function will be performed before, during and after each HepaStem infusion for all the patients based on vital signs** (See Section 6). A continuous pulse oximetric monitoring of blood oxygen saturation, with heart rate, respiratory rate and blood pressure measurements will be performed during HepaStem infusions. Any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement.

The next main organs where the cells are expected to migrate are liver and spleen. Cirrhotic patients are known to have portal hypertension, which potentially reduces portal arterial and venous flows. In addition, cirrhotic patients have coagulation disturbances with increased risks of bleeding and portal vein thrombosis (see Section 1). **On each treatment day before HepaStem infusion, hepatic ultrasonography and Doppler ultrasound exams will be performed.** The exam will include examination of liver parenchyma, and at least the main portal veins and branches, hepatic artery (hepatic artery resitivity index) and other hepatic vessels including flow direction, flow velocity. Patients in whom portal flow cannot be seen will have a repeated exam within 24 hours and/or additionnal exams if needed (abdominal CT scan or MRI) to exclude thrombosis of the portal vein. **Hepastem will be administred only if portal vein patency is demonstrated (excluding thrombosis of the portal vein).**

5.5.2. Transfusion like reaction

Infusion of biologic products may lead to transfusion like reaction, with symptoms such as fever, chills, CRP increase. This can be treated with corticoids such as methylprednisolone. As a preventive measure, a bolus of hydrocortisone or equivalent will be given 15 to 30 minutes before each HepaStem infusion (see Section 5.6).

5.5.3. Allergic Reaction

Infusion of biologic products may lead to allergic reaction. The signs and symptoms include: urticarial, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema.

5.5.4. AEs Related to Vascular Access

Catheter infection, pneumothorax, hemothorax, bleeding at the site of insertion and thrombosis can occur in patients with central line catheters. Catheters will be inserted and manipulated by specialized personal to minimize the risks for these complications.

5.5.5. Risk of immune response and rejection

The development of allogenic immune response following HepaStem infusion may reduce HepaStem efficacy although limited safety risk are expected. Anti-HLA antibodies will be measured in order to document the patient potential allogenic response.

5.5.6. Theoretical potential risk of tumor formation

Risk of tumor formation has not been reported in association with mesenchymal stem cell therapy. HepaStem is a progenitor cell presenting signs of pre-differenciation and no evidence of tumorogenicity was observed with HHLAPC: when expended *in vitro* until cell death, cells entered into senescence; no tumor formation was observed in immunodeficient mice for up to 90 days or 24 weeks post-administration.

In this study, after the active study period of 28 days, patients will have a Safety follow-up period up to 1 year after D1 (first HepaStem infusion)

Thereafter, patients will be invited to be followed-up in the Patient long-term safety follow-up Registry for 5 additionnal years

5.6. CONCOMITANT TREATMENTS

All patients will receive standard medical treatment (SMT) as required by their clinical status.

A single bolus of 100 mg hydrocortisone will be given 15 to 30 minutes before the HepaStem infusion .

All efforts should be made to use 100 mg of Hydrocortisone according to the above-mentioned protocol. Nevertheless, if 100 mg hydrocortisone is not available, the following equivalent can be accepted:

Compound	***Equivalent Dose***	***Biological Half-life***
Cortisone	125 mg	Short (8-12 hours)
Hydrocortisone	100 mg	Short (8-12 hours)
Prednisolone	25 mg	Intermediate (12-36 hours)
Methylprednisolone	20 mg	Intermediate (12-36 hours)

The exact treatment administrated should be documented in the Source Document and in the eCRF.

Patients are requested to accept abstinence from alcohol during the active study period (day 28).

6. STUDY CONDUCT

6.1. STUDY ACTIVITIES

6.2. STUDY VISITS

During the screening and treatment period, patients will be hospitalised in intermediate or ICUs or standard units, depending of the severity of the patient disease.

During the infusion of the treatment, the patient should be monitored in a specific unit to allow a continuous monitoring of his status (depending on the organization of the site, this specific unit can be a specific bed to ensure continuous monitoring of the patient, clinical Investigationnal Unit, Intensive Care Unit or Continus monitoring Unit).

Once informed consent is signed, the screening period may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation of cirrhosis and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation of cirrhosis and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach as described in Section 3.1

On HepaStem infusion days, before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, coagulation factors, TEG (if already performed as part of the clinical routine and up to investigator's judgment) a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurily stopped.

The careful evaluation of these parameters will allow or not the infusion (see section 4.4 – Criteria for treatment discontinuation).

During the infusion, the patient will be continuously monitored for early detection of any potential AEs.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

A study visit will be performed on Day 14 $\pm$ 2 days, including the evaluations listed below.

In case of any suspicion of AE, the investigator will perform the exams she/he evaluates as appropriate. In particular, any change or worsening in blood oxygen saturation should lead to perform an arterial blood gas measurement. In case of clinical signs or suspicion of pulmonary embolism, the investigator should perfom exams she/he evaluates as appropriate such as pulmonary angio-scanner or pulmonary scintigrapy.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the long term follow-up period, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to the 28 days visit, all SAEs will be collected. After the 28 days visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, transplantation and outcome of the transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem (see Section 7.1.2).

At the Month 12 study visit, patients will be invited to be followed-up in the Patient long-term safety follow-up Registry for 5 additional years.

6.2.1. Study assessments

- All AEs up to Day 28
- All AESI up to 1 Year.
- Concomitant medication modifications up to Day 28
- Physical examination: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
- Vital signs (Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation) :
    - At screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - Before, during and after infusion
- West-Haven HE, CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD score and Child Pugh will be estimated from clinical and biological results: at screening, on Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3. 6 and 12
- Common clinical laboratory tests: at screening, Days 1, 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, Red Blood Cell count, platelets [on infusion day, the platelets will be measured prior and post infusion at 4h, 24h, 48h and 72h].
    - GOT, GPT, bilirubin, alkaline phosphatase, gamma GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT
    - Serum albumin, sodium, potassium,
- Following coagulation factors will be closely followed prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2). In case of deterioration of the coagulation, the frequency of these exams can be increased up to the investigator's judgment.
    - INR
    - aPTT

- fibrinogen
- D-Dimers
- TEG (optional, only if measurement can be done locally and up to investigator's judgment)

- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion.
- Lipase: at screening
- Fibrinogen at screening
- Viral serology (HIV, HCV, HEV, HbS antigen) and aspergilus detection: at screening (if not performed during same admission)
- Urine test (Sediment, Creat, Glc, Protein, Albm): at screening
- Protein C, Protein S, anti-thrombin III: at screening. Thomboelastogram, thrombin generation test: at screening, Days 1 (before infusion and 4h post infusion), 4, 8 (before infusion and 4h post infusion for patients in cohort 2), 12, 14, 21, 28 (blood testing in central lab)
- Anti-HLA antibodies (class I and class II by Luminex™ method): at screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at screening, Days 1, 8, 14 and 28 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, before each infusion on Days 1, 4, 8, 12 and on Days 14 and 28 and also on M12
- Chest x-ray at screening (if not performed during same admission) and at M12,
- Blood culture, other fluid culture: if applicable at screening (If already performed during same admission, results collected)
- ECG: at screening (if not performed during same admission)
- Cardiac echography and Doppler: at screening (if not performed during same admission), on Day 1 after infusion and at M12

A SMC will review safety data and advice on study conduct as described in Section 3.1 and Section 9.13.

Additional visits including remote visit, phone calls after hospital discharge, could be performed and reported in the eCRF (floating visit) at the investigator's discretion.

In case of premature withdraw from study, an end of study visit should be perfomed if possible at the time of study withdraw.

In case of liver transplantation, a sample of the explanted liver will be collected if possible.

6.2.2. Central Blood sampling

Several tests will be performed in central labs. Further information will be provided in the Lab Procedure Manual.

<u>Thromboelastogram and thromboglobulin generation tests</u>

Blood will be collected on citrated tube fully fulled-in. Blood must be centrifuged within 30 min. A double centrifugation must be performed (2500 G for 10 min twice). The plasma will be collected and put in cryotubes (min. 2 tubes containing each min. 500 µL of citrated plasma), and immediately frozen (at -30°C for max. 3 weeks or at -80°C for max. 6 months).

Cliniques Universitaires St LUC
Laboratoire d'Hémostase
Avenue Mounier, 53
1200 Woluwe-Saint Lambert BELGIUM

Anti-HLA antibodies

Serum will be collected and send within 48 hours (ambient temperature) to :

Prof. Dominique Latinne
St Luc Hospital –Tour Franklin
Avenue Mounier 53, 1200 BRUSSELS – BELGIUM.

Plasma cytokines

Four mL of bloof will be collected on EDTA tubes. The blood will be centrifuged rapidly after sampling and plasma will be kept at -80 °C.

TARGID/Hepatology Department of Clinical and Experimental Medicine
Laboratory of Hepatology KU Leuven
Herestraat 49
B-3000 LEUVEN
BELGIUM

6.3. STUDY FLOW CHARTS

Table 6-1 Study Flowchart

Period	**Screening Period**	**Active period**										
	Baseline	**Treatment Period**					**Surveillance Period**		***Long term follow-up***			
Time	**Over 1-7 days prior D1**	Infusion D1	D4 ± 2 days	D8 ± 2 days	D12 ± 2 days	D14 ± 2 days	**D21** ± 2 days	**D28** ± 2 days	**M2** ± 2 weeks	**M3** ± 2 weeks	**M6** ± 2 weeks	**M12** ± 1 month
Informed Consent	X											
Eligibility criteria	X											
Demography & Medical History	X											
Physical exam	X	Xa	X	Xa	X	X	X	X	X	X	X	X
Vital Sign	X	Xb	X	Xb	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD, MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	X%	X	X%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	X%	X	X%	X	X	X	X	X	X	X	X
Lipase & Coagulation 2: C-protein, S-protein, Anti-Thrombin III	X											
Coagulation 1 : INR, aPTT	X	X+	X	X+	X	X	X	X	X	X	X	X
Virology status (HbS Ag, HCV, HEV, HIV), Aspergilosis test	X											
Coagulation 3 : Fibrinogen	X	X+		X+								
Coagulation 3 : D-Dimers, optional local TEG		X+		X+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	Xa		Xa		X		X				
TEG, TG	X	X*	X	X*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	Xa	X	Xa	X	X	X	X				X
Chest X-Ray	©											X
ECG	©											
Cardiac US Doppler	©	≠										X
Blood culture or other fluid culture	c											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶ cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X										
Cohort 2a & b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2b only								
Cohort 2c & d : Infusion of 1.0.10⁶ cells /kg body weight with a maximum of 100.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2d only								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a: On infusion day: before infusion	b: On infusion day: before, during and after infusion
%: On infusion day: all parameters are measured prior infusion/platelets measurement to be performed prior and post infusion at 4h, 24h, 48h & 72h	C: Only if performed during the same admission
+: On infusion day: prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion	&: On infusion day: prior and 24h after infusion
©: if not already performed during same admission. If already performed, results collected	*: On infusion day: prior and 4h after infusion
AESI: Only Adverse Event of Special Interest to be reported	≠: cardiac US to be performed after infusion

7. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

7.1. DEFINITIONS

7.1.1. AEs and ADRs

An *Adverse Event (AE)* is defined in ICH Guideline for GCP as "any untoward medical occurrence in a patient or clinical investigation Patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose.

Interventions for pre-existing conditions or medical procedures planned prior to study enrollment are not considered AEs. For the purpose of this study, AEs will be collected after informed consent signature.

Adverse Drug Reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose where a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An *unexpected drug reaction* is an ADR, the nature and severity of which is not consistent with the applicable product information, e.g. the Reference Safety Information in the Investigator Brochure.

In addition to constant observation of the trial Patient for his/her well-being, the Investigator will question the trial Patient regarding the occurrence of any AEs at each visit without exerting any influence on the Patient by the way of questioning.

For all AEs, the Investigator must pursue and obtain enough information to determine the outcome of the AE and to assess if the criteria for classification as a serious adverse event (SAE) is met (see below), which requires immediate notification to Promethera Biosciences. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the Investigator is required until the event resolves or stabilizes at a level acceptable to the Investigator and the clinical monitor of Promethera Biosciences.

Until visit day 28 inclusive, all AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF regardless of the causal relationship to study drug.

7.1.2. Adverse Event of Special Interest (AESI)

For the 11-month follow-up extension, only Adverse Event of Special Interest (AESI) will be collected. The AESI are events potentially associated with the investigational compound or disease under study. The Adverse Event of Special Interest are defined as:

- AEs with fatal outcome

- Transplantation and outcome of the transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

They will be considered and reported as SAEs.

7.1.3. SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the Patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (ie, severe allergic reaction, malignancy).

A planned hospitalization for pre-existing condition or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be an SAE (e.g. prolonged hospitalization after cell infusion due to family situation). However, re-occurrence of ACLF after end of infusion period and before final visit, if responsible for prolongation of hospitalization, is a SAE.

Until visit day 28 inclusive, any SAE occurring during the study must be reported, whether considered treatment-related or not. A life threatening event is any event in which the trial Patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This does not include an event that, had it occurred in a more serious form, might have caused death.

Until visit day 28 inclusive, all SAEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records, in the eCRF and on the SAE report (SAER) form, regardless of the causal relationship to study drug.

7.1.4. Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is any event that is serious as per the above criteria, the nature or severity of which is not consistent with the applicable product information (e.g. IB) and the causal relationship to the study drug is judged by the Investigator or Promethera Biosciences to be possible, probable or definite.

7.1.5. Serious Adverse Drug Reactions (SADR)

A SADR is any ADR that is serious as per the above criteria.

7.2. AE REPORTING PROCEDURES

All AEs observed by the Investigator or voluntarily reported by the patients enrolled into this study must be collected and recorded by the Investigator in the Patients' medical records and in the eCRF. Hence:

AEs and ADRs will be collected as of the day ICF signature. Clinical monitor will list all AEs and provide it to Promethera Biosciences on a regular basis as part of the visit report. AEs reported before screening visits will be assessed as part of medical history.

7.2.1. Assessment of AEs

Documentation of the AEs in the eCRF will be performed according to the following criteria:

- Description of the AE: diagnosis if known, with signs and symptoms, giving appropriate details to the event
- Date and time of the onset, and resolution of the AE
- Severity, graded as in Section 7.2.3
- Assessment of causal relationship to study treatment, as in Section 7.2.2
- Action taken regarding study treatment and treatment given
- Outcome: complete recovery/not yet recovered/recovered with sequelae/death/unknown.

The Investigator will be asked to provide follow-up information and/or discharge summaries as needed.

7.2.2. Assessment of Causal Relationship

The relationship of the AE will be assessed following the definitions below:

<u>Unrelated</u>

"Clearly not related"

Any event that does not follow a reasonable sequential order from administration of study drug and that is likely to have been produced by the trial Patient's clinical state, therapeutic interventions or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. Reports contain satisfactory information that the AE is not related to the study treatment.

<u>Unlikely</u>

"Doubtfully related"

Any event that does not follow a reasonable sequential order from administration of study drug or that is likely to have been produced by the trial Patient's clinical state or other modes of therapy administered to the Patient and that does not follow a known response pattern to the study drug. The causal relationship to the study treatment cannot be excluded beyond reasonable doubt, but the reports contain reasons that the AE is most likely caused by other factors than the study treatment.

<u>Possibly</u>

"May be related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug and that could readily have been produced by a number of other factors. Even though the relationship of the study drug to the AE may be uncertain or doubtful (e.g. due to missing data or insufficient evidence), the possibility of a causal relationship exists.

Probably

"Likely related"

Any reaction that follows a reasonable sequential order from administration of study drug or that follows a known response pattern to the suspected drug; reports contain satisfactory information to assume a causal relationship to the study treatment and the AE could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

Definitly

"Clearly related"

Any reaction that follows a reasonable sequential order from administration of study drug, follows a known response pattern to the suspected drug that improves upon reducing the dose or stopping the study treatment and recurs on repeated exposure, and that could not be reasonably explained by the known characteristics of the trial Patient's clinical state or other modes of therapy administered to the Patient.

7.2.3. Severity of AEs

AEs will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)[1];

Moderate, when interfering with ADL;

Severe, when impeding ADL.

Severity must be distinguished from seriousness, which is based on the consequence of the AE. For example, a headache may be mild, moderate or severe, though rarely is it serious.

7.2.4. SAE Reporting Procedures

SAEs/SADEs will be collected as of the day of informed consent for study participation is provided.

[1] Self-care ADL: bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

The EU Directive 2001/20/EC on clinical trials defines all the legal requirements with regards to pharmacovigilance in clinical trials in Europe. It requires Investigators to report all SAEs immediately to the Sponsor who is responsible of maintaining detailed records of all reported AEs.

Hence, the Investigator is responsible for reporting all SAEs to Promethera Biosciences within 24 hours of discovery. The immediate SAE reports give information on the type (death; life threatening; requires or prolongs in-patient hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defect) and description of the event, causal relationship to the study drug, number of received HepaStem infusions, date of last infusion, anonimous identification of the trial Patient, trial and Investigator.

Initial SAE reports will be followed by relevant detailed medical records as soon as they become available, and autopsy reports should be provided for deaths if available. The Investigator must ensure that the trial Patient's anonymity is maintained. The trial Patient's name should be replaced by the screening and/or patient number on all reported copies of hospital documents and reports.

Article 17 of The EU Directive 2001/20/EC requires that each SUSAR from all clinical trials on a particular investigational medicinal product must be reported electronically to the EMA pharmacovigilance database, EudraVigilance Clinical Trial Module (EVCTM).

To be classified as a SUSAR, the SAE should have a causal relationship to study treatment that is judged by the Investigator or Promethera Biosciences to be possibly, probable or definite.

Determination of expectedness is based on the contents of the latest version of the IB.

Promethera Biosciences is responsible to report on an expedited basis to Competent Authorities (CA) of the concerned member states and to the IEC all relevant information about SUSARs. Timelines for reporting are specified: fatal and life threatening SUSARs have to be reported 7 days from the date of initial report, and an additional 8 days for relevant follow-up. All other SUSARS shall be reported in a maximum of 15 days.

Once a year, Promethera Biosciences shall provide CA and IECs with a listing of all Serious Adverse Reactions (SARs) and a report of the Patients' safety, the Annual Safety Report.

7.2.5. AESI Reporting Procedures

Adverse Event of Special Interest as defined in the part 7.1.2 will follow SAE reporting procedure defined in the part 7.2.4.

7.2.6. Pregnancy

Female patients who are pregnant will not be allowed to participate in the study. A blood test must be performed at screening on all females of child bearing patiential. Women of child bearing potential should employ effective contraceptive methods during HepaStem study. Intrauterine contraceptive devices, etonorgestrel implants, barrier methods, or abstinence are acceptable.

The investigator is responsible for reporting any pregnancy to Promethera Biosciences within 24 hours of discovery. All pregnancy observed by the investigator or voluntarily reported by the patients

enrolled into this study must be collected and recorded by the investigator in the Patients' medical records, and in the CRF.

7.2.7. Follow-up of AEs

All AEs and SAEs should be followed up by the Investigator until resolution or until the degree of persistent disability can be assessed. Adequate medical care shall be provided to the study Patients in case of an AE or an SAE.

For SUSARs, the follow-up should include detailed investigations until a clinical outcome is reached and final conclusions and any corrective and preventive measures are identified, including confirmation of whether it was an SADR.

8. STATISTICAL METHODS

8.1. STUDY DESIGN

This is an interventional, multicenter, open, safety study of different dose regimens of HepaStem given in subsequent cohorts each with approximately 21hospitalized patients in total.

For detailed description of the primary and secondary endpoints, please refer to Section 3.2

8.2. SAMPLE SIZE

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile. In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population. Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

The 3 first patients infused (cohort 1a) : 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period ($\pm$ 2 days); at least 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone or equivalent was given 15 to 30 min before each HepaStem infusion.

2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage (see section 1).

Approximately 3 patients will receive a lower dose of HepaStem (in cohort 1b) and approximately 15 patients will receive the higher dose in cohort 2 (cohort 2a + 2b + 2c + 2d).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Since it is a safety study, any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

In total approximately 21 evaluable patients will be included. The total sample size will depend on the recommendations given by SMC in order to protect patient safety based on a risk assessment.

8.3. STATISTICAL ANALYSIS

8.3.1. General Considerations

Statistical analysis of this study will be the responsibility of Promethera Biosciences or its designee.

More details about statistical methods and data to be reported will be described in the Statistical Analysis Plan (SAP).

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics, taking into account the doses applied.

Categorical endpoints will be summarized by the number of Patients, frequency, and percentages of each category.

Missing values will not be imputed.

8.3.2. Study Participant Disposition

All Patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their study discontinuation will be given.

8.3.3. Analysis

All statistical analyses will be based on the Included Population defined as the subset of patients who receive at least one infusion.

All study variables will be listed by treatment dose and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Patients' demographic and baseline characteristics, patient disposition, extent of exposure, the number and proportion of patients who complete all planned infusions, the number and proportion of patients who discontinued treatment and the reasons for premature discontinuation of treatment will be summarised by treatment dose and overall.

All clinical data, vital signs, laboratory data, portal and liver echography and Doppler ultrasound together with the corresponding changes from baseline (values at screening), and baseline examinations will be summarised by treatment dose and overall. Worsening changes will be assessed for their clinical significance by study investigators. If considered as clinically significant, they will be reported as AEs.

AEs and SAEs will be summarized by treatment dose and overall, type, incidence, severity, seriousness, and relationship to treatment. The relationship will be assessed based on investigator assessment. An additional relationship assessment based on global review of AEs will be performed by the SMC and sponsor pharmacovigilance.

Disease scores, bilirubin, creatinine, INR and albumin values and the corresponding changes from baseline (values at screening) will be summarized by treatment dose and overall. Global and individual changes in comparison to baseline status will be reported.

Adverse Events of special interest (AE with fatal outcome, transplantation and outcome of thetransplantation, onset of malignancies, hospitalization for ACLF, AEs assessed by the investigator as possibly related to HepaStem)) will be listed and summarised by treatment dose and overall.

The first analysis will be performed after first 12 treated patients of cohorts 1 and 2 will have completed the 28-day active study period or have died or have been lost to follow-up.

The second analysis will be performed after these patients will have completed the 3-month follow-up or have died or have been lost to follow-up.

A third analysis will be performed after all (approximately) 21 patients will have completed the 28-day active study period or have died or have been lost to follow-up.

The fourth analysis will be performed after all (approximately) 21 patients will have completed the 3-month follow-up or have died or have been lost to follow-up.

The fifth and final analysis will be performed after these approximately 21 patients will have completed the 1-year follow-up or have died or have been lost to follow-up.

8.3.1. Study reports

The first study report will be produced after first 12 treated patients of cohorts 1 and 2 will have completed the 28 day active study period or have died or have been lost to follow-up.

The report will be updated after these patients will have completed the 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after all (approximately) 21 patients will have completed the 28 day active study period and 3 month follow-up or have died or have been lost to follow-up.

The report will be updated after all approximately 21 patients will have completed the 1 year follow-up or have died or have been lost to follow-up.

9. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1. PATIENTS' INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to ensure that no patient is Patient to any study related procedures before having given written informed consent that has been previously approved by the relevant independent Ethics committee (IEC) in each participating country.

The patient population in this study will be represented by male or female patients of minimum adult legal age (according to local laws for signing the informed consent document), able to provide written informed consent, and able to understand and comply with the requirements of the study. In patients with hepatic encephalopathy [HE], if the patient is not able to understand the study based on the investigator's judgment, the informed consent may be obtained from a patient's representative and confirmed by the patients after resolution of the impairment in brain function. If the Patient is unable, the representative must sign the consent form. The Patient should also do so as far as possible. The Patient will not be able to participate until he/she (and/or the representative) signs the consent form.

The Patient (and/or patient's representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the Patient's data and information.

The Investigator or a physician delegated by the Investigator, in accordance with GCP-ICH, shall give information on the purpose of the trial and its nature, explain the potential benefits and risks in detail and give assurance that the treatment shall not be prejudiced in case participation in the trial is refused, or consent is withdrawn at any point during the trial. The Investigator shall make certain that the information has been understood and that there has been enough time allowed to give an informed decision. The Investigator shall not take part in decision making.

The receipt of the informed consent will be documented in source documents and in the eCRF. Two originals will be completed and one copy of the consent form signed and dated by the patient (and/or representative) and by the physician who informed the patient (and/or representative) will be handed over to the patient (and/or representative). A second copy will be kept at the study center. Each patient will receive a patient information sheet written in local language.

9.2. ETHICAL CONDUCT OF THE STUDY AND IEC APPROVAL

This protocol accords with the principles of the Declaration of Helsinki as set forth at the 18th World Medicines Association (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989), the 48th (Somerset West 1996), and the 52nd (Edinburgh 2000), and most recently the 64th World Medicines Association in Fortaleza 2013. As these accords are reviewed and amended periodically, the most current amended version will be in effect.

The written approvals of the CAs and relevant IECs must be obtained and the national regulatory requirements of the respective participating country must be fulfilled before a study center is initiated. Prior to implementing any changes in the study, Promethera Biosciences will produce the relevant

protocol amendment and these amendments will also be submitted to the national authorities and relevant IECs for approval.

On the approval letter, the study (title, protocol number and version), the documents reviewed (protocol, IB, informed consent material, amendments) and the date of review should be clearly stated. The patient recruitment will not begin until this written approval has been received by Promethera Biosciences.

9.3. INVESTIGATOR RESPONSIBILITIES

The Investigator must perform the study in accordance with the current approved protocol, the principles of the Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements. A copy of the ICH-GCP guidelines will be available in the Investigator Site File.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the investigational site prior to commitment to participate in this study.

The Investigator shall maintain a list of appropriately qualified persons to whom significant study-related tasks are delegated. A signed copy of the curriculum vitae (not older than 2 years) for the Investigator and sub-Investigator(s) will be provided to Promethera Biosciences prior to the start of the study. The Investigator will inform Promethera Biosciences of any updates to the study team list.

If the patient has a primary physician, the Investigator should, with the patient's consent, inform the physician of the patient's participation in the study.

The Investigator must adhere to the protocol as detailed in this document.

The Investigator will be required to sign an Investigator agreement to confirm acceptance and willingness to comply with the study protocol.

The Investigator shall be responsible for enrolling only those patients who have met the protocol eligibility criteria.

It is the responsibility of the Investigator to ensure that all appropriate approvals are in place prior to recruitment.

The Investigator should notify the IEC of any SAEs occurring at the site and other AE reports received from Promethera Biosciences, in accordance with local procedures and regulations.

Agreement with the final clinical study report will be documented by the signed and dated signature of the principal or coordinating Investigator in compliance with ICH E3.

9.4. CONFIDENTIALITY OF PATIENT RECORDS, TRIAL DOCUMENTATION AND DATA

Data protection consent must be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative in accordance with the ICH GCP and the applicable privacy requirements (e.g. European Union Data Protection Directive 95/46/EC ["EU Directive"] and any other country privacy requirements). According to ICH-GCP guideline, Promethera Biosciences

must “verify that each Patient has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IEC review, and regulatory inspection”.

Neither the names of the patients nor any other records identifying the Patients will be made publicly available by any of the parties authorized to review the data.

The Investigator must ensure that the anonymity of each patient is strictly maintained. On CRFs or other documents submitted to Promethera Biosciences, the patient will be identified by a code, namely screening number and/or patient number. A separate patient identification list of these codes will be kept in the Investigator Site File. This information will not be submitted to Promethera Biosciences. Completed consent forms shall be kept in the Investigator Site File in strict confidence.

After patients, or if not capable, their representative have consented to take part in the study, the medical records and the data collected during the study will be reviewed by representatives of Promethera Biosciences and/or the company organizing the research on Promethera Biosciences behalf to confirm that the data collected are accurate and are collected for the purpose of analyzing the results. These records and data may additionally be reviewed by auditors or by regulatory authorities.

Data and results of this clinical study are the sole property of Promethera Biosciences and may be used to support the development and/or registration of HepaStem. All data collected during this study will be controlled by Promethera Biosciences or designee, and Promethera Biosciences will abide by all relevant data protection laws.

9.5. PATIENT INSURANCE

Patients included in this clinical study are Patient to a patient insurance signed by Promethera Biosciences. In order to be able to benefit from this insurance, the patient is obliged to comply with the stipulations accepted in his/her written informed consent.

The Insurance Certificate and the provision clauses will be filed in the Investigator’s Site File.

9.6. MODIFICATION OF THE PROTOCOL

Any change to the protocol can only be made as a written amendment to the trial protocol. The written amendment, signed by the Coordinating Investigators and Promethera Biosciences, will be submitted to all the relevant IECs.

If needed, patient information and ICF documents must then be amended accordingly and the amended ICF must be signed by the patient (or representative) if the changes affect the patient’s further participation in the study.

9.7. PROTOCOL DEVIATIONS

9.7.1. Site Staff Awareness

During the SIV, the site staff should be trained on the procedures and requirements of the protocol. The procedure for the handling of the protocol deviations should be explained. The following items should be discussed during this visit:

- No deviation or change from the protocol may be implemented without the written agreement from Promethera Biosciences;
- Documented approval from the EC must be present, except for eliminating immediate hazard(s) to trial Patients. In such a case, prior approval is not necessary, but Promethera Biosciences and the EC must be informed about the deviation as soon as possible;

Any deviation from the protocol must be documented and explained.

9.7.2. Handling and Reporting of Protocol Deviations

The monitor will verify the compliance and will ensure that any protocol deviation discovered during a monitoring visit is reviewed and discussed with the site staff. Possible reason(s) for the deviation(s) should be discussed and corrected, and preventive actions should be formulated if needed.

Each major protocol deviation should be recorded on the "Protocol Deviation Form" (PDF) and needs to be countersigned by the Principal Investigator. The original PDF will be collected and stored in the Investigator Office File and in the Trial Master File. A copy will also be filed at the site.

The monitor will report any newly discovered protocol deviations in the Monitoring Visit Report (MVR). In addition, any discussion related to proposed changes to the protocol formulated by the investigator, will be communicated to Promethera Biosciences and will be recorded in the MVR.

Promethera Biosciences will review all protocol deviations at a minimum on a yearly basis in order to assess possible trending. Additional reviews can be performed based on the actual number of protocol deviations that are reported during the course of a trial. Promethera Biosciences will be informed immediately by the monitor in case any serious and/or persistent non-compliance issues identified at a site.

Promethera Biosciences will evaluate serious or persistent non-compliance, and a corrective or preventive action plan will be put in place. In this case, the Principal Investigator will be contacted by Promethera Biosciences to prevent the non-compliance from recurring. If the non-compliance is persistent or leads to a serious safety hazard for the Patient, the ethics committee and all applicable regulatory bodies will be informed.

9.8. STUDY MONITORING

Promethera Biosciences' monitor is responsible for monitoring the progress of the clinical investigation and verifies the reported data at the investigational site(s), with the aim to ensure compliance with the investigational plan, ICH-GCP and applicable regulations, protect the rights and safety of patients, safeguard the quality and integrity of the reported data, updates Promethera Biosciences on the status and performance of the investigational sites.

During the MV, the monitor will verify that:

- Compliance with the protocol is maintained and any deviation is discussed with the investigator, and is documented and reported to Promethera Biosciences;
- The investigational product is being used according to the protocol. Promethera Biosciences will be informed as soon as possible if modifications are requested, either to the investigational product or the protocol;
- The investigator has and continues to have sufficient staff and facilities to conduct the investigation safely and effectively;
- Any changes in staff have been documented in the Delegation Log, CVs have been collected for any new staff and appropriate training has been documented.
- The investigator has and continues to have access to an adequate number of Patients;
- The investigator has and continues to have sufficient study materials on site (eCRFs, investigational products, etc.);
- Signed and dated informed consent has been obtained from each Patient or legal representative prior to any study related procedure;
- The reported data in the eCRFs is accurate and is consistent with the source documents;
- The procedures for recording and reporting adverse events and adverse device/drug effects to Promethera Biosciences are followed;
- Patient withdrawal and/or non-compliance is documented and discussed with the investigator, and is reported to Promethera Biosciences after the visit;
- Investigational product storage, according to the instructions for use, should be reviewed and accountability performed;
- The Investigator Office File and Investigator Site File are up-to-date.

Queries may be raised if the data is unclear or contradictory. These queries must be addressed by the Investigator or delegate as stated in the study staff log.

9.9. ACCESS TO SOURCE DATA

All data must be recorded in the patient's hospital notes/medical chart.

The monitor will have access to the patients' medical records and other study-related records needed to verify the entries in the eCRFs.

In addition to demographic data, medical history and relevant investigations/lab reports etc, the source document (the original patient file) should clearly state the date of entry in the trial, the trial protocol number, date of consent form signature, date of each trial visit, date and time of all study related measurements, applications, AEs and changes in the concomitant medications. In case of withdrawal of the patient from the study, the reason must be indicated, and at least the date of study termination recorded.

The Investigator will permit authorized representatives of Promethera Biosciences, the respective health authorities, and auditors to inspect facilities and records relevant to this study.

The monitor and/or auditors and/or regulatory inspectors will check the eCRF entries against the source documents. The consent form will include a statement by which the patients allow the monitor/auditor/inspector from the IECs or regulatory authorities access to source data (e.g. patient's medical file, appointment books, original laboratory test reports) that substantiate information in the eCRFs. These personnel, bound by professional secrecy, will not disclose any personal information.

9.10. CASE REPORT FORMS

Electronic CRFs that have been designed to record all observations and other data specific to the clinical trial will be provided by Promethera Biosciences.

The Investigator is responsible for maintaining adequate and accurate eCRFs. Each eCRF should be filled out completely by the Investigator or delegate as stated in the study staff log.

The eCRFs should be reviewed, signed and dated by the Investigator.

9.11. ARCHIVING

The Investigator is responsible to archive all "essential documents", as described in the ICH GCP Guidelines, including eCRFs, source documents, consent forms, laboratory test results, and drug inventory records until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Promethera Biosciences.

Prior to the destruction of any study document, the Investigator should obtain written permission from Promethera Biosciences. These records should be made available at reasonable times for inspection by the Regulatory Authorities.

9.12. PUBLICATION OF RESULTS

The data and the results of this clinical study are the sole property of Promethera Biosciences. Promethera Biosciences retains the right to review all material prior to presentation or submission for publication. No party is permitted to publish/present the results of the study, in part or in their entirety, without the written authorization of Promethera Biosciences.

9.13. SAFETY MONITORING COMMITTEE (SMC)

The Safety Monotoring Committee will be composed of members with expertise in liver disease or other relevant medical fields, all external and independent to Promethera. The members of the SMC provide their expertise and recommendations.

The primary responsibilities of the SMC are:

1. Periodically review (at specified timepoints) and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.

2. Make recommendations to Promethera Biosciences regarding the continuation, modification, or termination of the trial. The SMC considers study-specific data as well as relevant background knowledge about the disease, investigational product, or patient population under study.
3. The SMC is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review. The SMC is also responsible for maintaining the confidentiality of the data reviewed and the reports produced.
4. The SMC will ensure a continuous supervision of the treatment stepwise approach as described in section 3.1. The low dose regimen will be given to the first cohort. Thereafter, the high dose regimen will be given to the second cohort.

As a minimum, the safety data will be reviewed by the SMC at the following timepoints:

- For each cohort (1b, 2a, 2b, 2c and 2d), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- For the first cohort (1b), when the second evaluable patient has received HepaStem infusion (complete scheme or premature stop), the patient data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme of premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

If needed, the frequency of the safety data review can be increased to early identify any potential risk and to re-evaluate the benefit/risk balance for the patient.

In case a same serious adverse event with a plausible causal relationship to HepaStem appears for more than 1 patient in the low dose cohort, the passage to the higher dose won't be authorized.

More specifically, based on the patient's parameters in cohort 2a, the SMC might also consider that the next patients will receive 2 infusions 1 week apart of a split dose of HepaStem ($0.25.10^6$ cells/kg body weight) instead of a unique administration of $0.5.10^6$ cells/kg body weight).

5. The SMC will review severe coagulation events assessed as related to HepaStem administration by the investigator.
6. At the end of the active study period, the SMC will review the AEs and will perform an evaluation of the biological plausibility of any causal relationship to HepaStem administration.

During each trial, the SMC should review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, SMC members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The SMC should also assess the performance of overall study operations and any other relevant issues, as necessary.

The SMC should conclude each review with their recommendations to Promethera Biosciences.

The membership of the SMC should reflect the disciplines and medical specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of SMC members depends on the phase of the trial, but generally consists of 3 to 7 members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied
- Expert(s) in hepatology
- Expert(s) in Homeostasis
- One or more biostatisticians
- Investigators with expertise in current clinical trials conduct and methodology.

Ad hoc specialists may be invited to participate as non-voting members at any time and Promethera Biosciences members may provide additional information if additional expertise is desired, but are not members of the SMC.

The frequency of SMC meetings will depend on several factors including the rate of enrollment, completion of patients in the dose group, safety issues or unanticipated AEs, availability of data, and, where relevant, scheduled interim analyses. The initial SMC meeting should occur preferably before the start of the trial or as soon thereafter as possible. At this meeting, the SMC should discuss the protocol and the SMC charter, which includes trigger set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines should also address stopping the study for safety concerns and, where relevant, for efficacy based on plans specified in the protocol. The SMC should also develop procedures for conducting business (e.g. voting rules, attendance, etc). Promethera Biosciences staff will discuss Promethera Biosciences' perspective on the study at this initial meeting.

Once a study is implemented, the SMC should convene as often as necessary to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed.

After each meeting of the SMC, the SMC's Chair should prepare a brief summary report. It should describe the SMC members' conclusions with respect to progress or need for modification of the protocol. These reports should be provided to the Investigators and to his/her local IEC and to Regulatory Authority if requested.

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11. APPENDIX 1 : SCORING SYSTEMS

11.1. CLIF ORGAN FAILURE (CLIF-OF) SCORE

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL, renal replacement therapy
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	 >300 >357	 ≤300 - > 200 >214- ≤357	 ≤200 ≤214

Arroyo et al. 2015

11.2. CLIF ACLF GRADE

ACLF grade	Organ failure
No ACLF	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from 1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

11.3. CLIF-C ACLF SCORE

CLIF-C ACLF = $10 \times [(0{,}33 \times \text{CLIF OF} + 0.04 \times \text{Age} + 0{,}63 \times \text{Ln}(\text{WBC}\{10^9 \text{ cells/L}\}) - 2]$

11.4. CLIF CONSORTIUM ACUTE DECOMPENSATION SCORE (CLIF-C AD)

CLIF-C AD = 10 × [(0,03 × Age {years} + 0,66 × Ln(Creatinine{mg/dL} + 1.71 × Ln(INR) + 0,88 × Ln(WBC{10^9 cells/L}) – 0,05 × Sodium {mmol/L} + 8]

Jalan et al. 2015

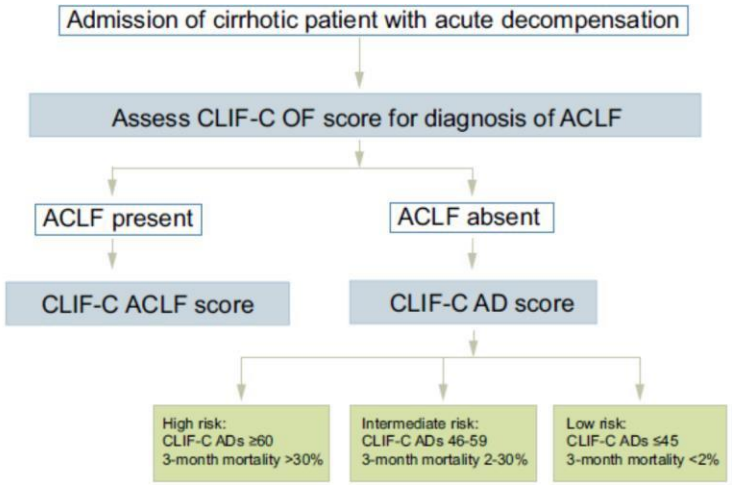

Fig. 4. Algorithm for the sequential use of the EASL-CLIF Consortium predictive scores in patients with cirrhosis admitted to hospital with acute decompensation.

11.5. MELD SCORE

MELD score is calculated using serum bilirubin, serum creatinine, and International Normalized Ratio (INR) and is given by the formula :

MELD(i) = (0.957 * ln(Serum Cr) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10 (if hemodialysis, value for Creatinine is automatically set to 4.0)

MELD Score (2016) = MELD(i) + 1.32*(137-Na) – [0.033*MELD(i)*(137-Na)]

Note: Sodium has a range of 125-137 mEq/L

11.6. THE SCORE CAN BE CALCULATED USING ONLINE WEBSITE HTTPS://WWW.MDCALC.COM/MELD-SCORE-MODEL-END-STAGE-LIVER-DISEASE-12-OLDERCHILD PUGH SCORE

Measure	1 point	2 points	3 points
Total bilirubin, μmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8

Prothrombin time prolongation (s) Or INR	<4.0 <1.7	4.0–6.0 1.7-2.3	> 6.0 >2.3
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	Class	One year survival	Two year survival
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

Singal AK, Kamath PS. Model for End-stage Liver Disease. J Clin Exp Hepatol. 2013 Mar;3(1):50-60. Review.

11.7. WEST HAVEN CRITERIA FOR HEPATIC ENCEPHALOPATHY

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	***Consciousness***	***Intellect and Behavior***	***Neurologic Findings***
0	*Normal*	*Normal*	*Normal examination; impaired psychomotor testing*
1	*Mild lack of awareness*	*Shortened attention span; impaired addition or subtraction*	*Mild asterixis or tremor*
2	*Lethargic*	*Disoriented; inappropriate behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
3	*Somnolent but arousable*	*Gross disorientation; bizarre behaviour*	*Muscular rigidity and clonus; Hyperreflexia*
4	*Coma*	*Coma*	*Decerebrate posturing*

12. APPENDIX 2: SIGNATURE PAGES

12.1. INVESTIGATOR'S SIGNATURE

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 05 Feb 2019

Version Number: 6.1

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

_____ _____

Signature: Date (dd/mm/yyyy):

12.2. SPONSOR SIGNATURES

Study Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

Study Number: HEP101

EudraCT No: 2016-001177-32

Protocol Date: 05 Feb 2019

Version Number: 6.1

The Clinical Study Protocol has been approved for submission to the Independent Ethics Committee and Competente Authorities and to conduct the Clinical Study in accordance with GCP-ICH by the following sponsor personnel who contributed to writing and/or approving this protocol:

Silver Ocean Ventures SAS, CEO, represented by John Tchelingerian Promethera Biosciences	Date
Etienne Sokal, Chief Scientific & Medical Officer Promethera Biosciences	Date

STATISTICAL ANALYSIS PLAN

HEP101 – PROMETHERA BIOSCIENCES

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Final version 1.0
05 December 2018

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1 List of abbreviations and definition of terms

Abbreviations	Definitions
KBP	Keyrus Biopharma
ICH	International Conference on Harmonisation
GPP	Good Pharmacoepidemiology Practices
FAS	Full analysis set
PP	Per protocol population
SAF	Safety population
SMC	Safety monitoring committee
TLG	Tables, Graphs and Listings

2 Introduction

This Statistical Analysis Plan (SAP) determines the frame of statistical analysis of this study in agreement with the protocol version 1.0 for Belgium dated on March 23nd 2016, the protocol version 2.0 for Belgium dated on December 13th 2016, the protocol version 3.2 dated May 11th 2017 for Belgium and version 4 dated February 15th 2018 in Bulgaria and Spain.

The SAP is reviewed, approved, and signed by the Biostatistician and Sponsor prior to database lock, at the latest.

This document will be the main reference document as far as statistical analyses are concerned.

The purpose of this document is to describe:

- The study features as per protocol in terms of objectives, study design and study conduct
- The endpoints, the study cohorts, the study variables and the derived data
- The planned statistical analysis and methodologies.

3 Study description

3.1 Study objective

3.1.1 Primary objectives

To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

3.1.2 Secondary objectives

- Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion.

- Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3.2 Study design

This is an interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total.

5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next three patients in cohort 1 (cohort 1b) received a lower dose (minimum ten times lower) in a single infusion ($0.25.10^6$ cells /kg bodyweight with a maximum of 25.10^6 cells per infusion).
3 patients in cohort 2 (cohort 2a received twice the dose of the patients in cohort 1b ($0.5.10^6$ cells/kg bodyweight with a maximum of 50.10^6 cells per infusion)).

3 patients in cohort 2 (cohort 2b) received up to 2 doses of $0.5.10^6$ cells/ kg bodyweight 1 week apart ($0.5.10^6$ cells/kg bodyweight per infusion with a maximum of 50.10^6 cells per infusion).

The study will recruit patients who are hospitalized for Acute Decompensation of cirrhosis or ACLF and/or who develop ACLF during hospitalisation.
The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

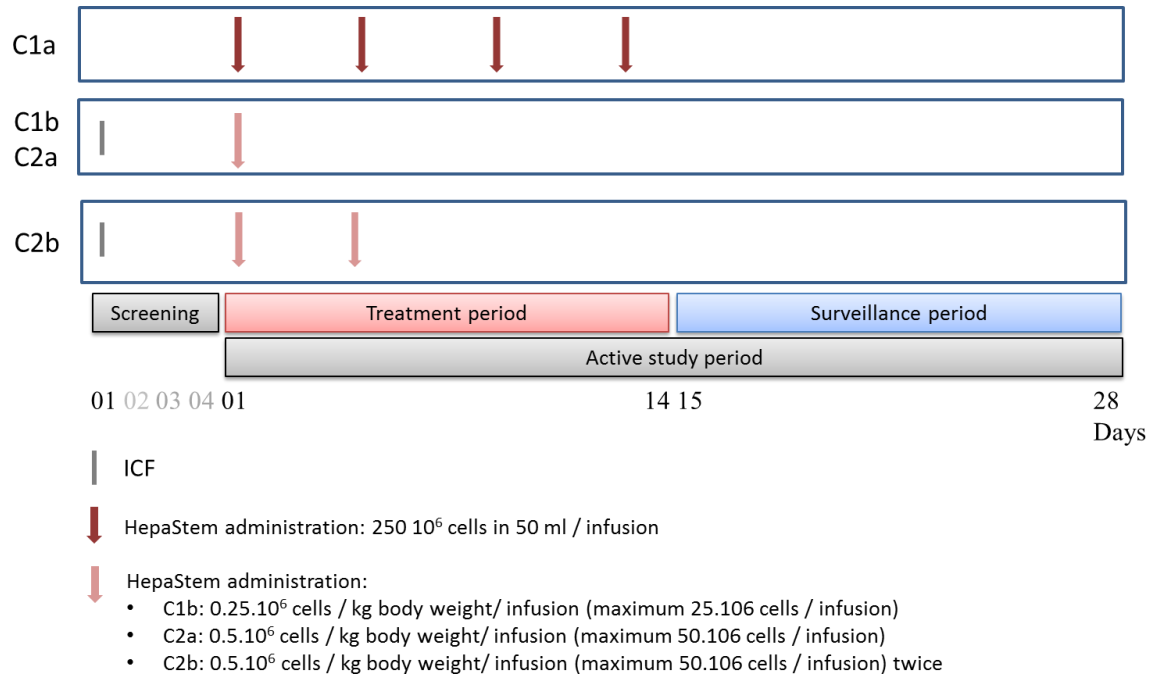
Screening period: Once informed consent is signed, may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days). The duration of the screening period plus the active period will last up to 35 days (±2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Various dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 1.

Figure1 -Study scheme of active study period



- Planned schedule:

For cohort 1a, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (+/- 2 days); at least a 2-day interval without infusion must be respected between infusion days.
In cohort 1a, 250 million cells in 50 ml were administrated on each infusion day, leading to a total of 1 billion cells if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone would be given 15 to 30 min before each HepaStem infusion.

- Actual schedule:

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (+/- 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem

suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

- Planned schedule based on protocol version 4:

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

For cohort 2a: 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of Hepastem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see Criteria for study treatment discontinuation)

Patients are treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of external members and Promethera members only for open session :

As a minimum, the safety data will be reviewed by the SMC at the following time points:

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be followed-up in the Patient Registry.

3.3 Study plan

During the screening and treatment period, patients will be hospitalised in intermediate or ICUs or standard units, depending of the severity of the patient disease.
Once informed consent is signed, the screening period may last from 1 to 4 days for protocol version 1, 2, 3.1 and 3.2 and up to 7 days for protocol version 4, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach.

During the treatment period, a study visit will be performed on Days 1, 4, 8, 12 and 14 (± 2 days for each visit form the Day 4 visit) including the evaluation listed in table 2.

On HepaStem infusion days before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, TEG (if already performed as a part of the clinical routine and up to investigator's judgment), a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurely stopped.

The careful evaluation of these parameters will allow or not the infusion or treatment discontinuation.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the - follow-up period up to 1 year, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to the 28 days visit, all SAEs will be collected. After the 28 days visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem .
At the Month 12 study visit, patients will be invited to be included in the long term safety follow up study (5- years).

Table 2 Study Flowchart

Period	Screening Period	Active period							Long term follow-up			
	Baseline	Treatment Period					Surveillance Period					
Time	Maximum 7 days prior D1	Infusion D1	D4[b]	D8[c]	D12[b]	D14[b]	D21[b]	D28[b]	M2[d]	M3[d]	M6[d]	M12[e]
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	X	«	X	X	X	X	X	X	X	X
Vital Sign	X	↔	X	↔	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	«[%]	X	«[%]	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	X	«	X	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	+	X	+	X	X	X	X	X	X	X	X
Coagulation 2 : C-Protein, S-Protein, Anti-Thrombin III	X[§]											
Virology status (HbS Ag, HCV, HEV, HIV), Aspergillosis test	X											
Coagulation 3 : Fibrinogen, D-Dimers, TEG®		+		+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	&		&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	«		«		X		X				
TEG, TG	X	*	X	*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	X	«	X	X	X	X				X
Chest X-Ray	©											X
Cardiac US Doppler	©	≠										X
ECG	©											
Blood culture or other fluid culture	A											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10[6] cells /kg body weight with a maximum of 25.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2a : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2b : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]		X[a]								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a) Hydrocortisone given 15-30 min before HepaStem infusion
b) ± 2 days
c) ± 2 days with at least 7 days interval without infusion
d) ± 2 weeks
e) ± 1 month
« Before each infusion.
A : If already performed during same admission, results collected
% : On infusion day, platelets measurement to be performed prior and post infusion at 4h, 24h, 48h and 72h[1].
© if not already performed during same admission; if already performed, results collected
↔ before, during, after each infusion
≠ : cardiac US to be performed after infusion
@ : Optionnal, only if measurement can be done locally and up to investigator's judgment
+ : On infusion day : prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion[2] (frequency of these exams can be increased up to the investigator's judgment.)
* : Before infusion and 4h post infusion[3]
AESI : only Adverse Event of Special Interest to be reported
TEG: Thromboelastogram, TG: Thrombin generation
& : Prior and 24h after infusion
§ : In case of deterioration of the coagulation, these measurements will be repeated.

[11] Modification of time point due to an error in the protocol version 4 (prior and post infusion at 1h, 24h, 48 and 72h)
[2] Modification of time points due to errors in the protocol version 4 (prior and 1h, 3h, 5h, 8h, 12h ,18h, 24 and 72h post infusion)
[3] Modification of time point due to an error in the protocol version 4 (before and 3h post infusion)

3.4 Changes in the conduct of the study

Cf; amendement

4 Statistical methods

4.1 General statistical considerations

All statistical analyses will be performed with the SAS software version 9.4.

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics by dose cohort.

Standard descriptive statistics will be used for quantitative and categorical variables.

Quantitative variables will be presented using the number of observed values, number of missing observations, mean, standard deviation, and median, minimum and maximum.
When required, Confidence intervals will be computed based on the Wald method.

Categorical variables will be presented using counts and percentages of patients. The number of missing observations will also be presented.
When required, Confidence intervals will be computed based on the Clopper-Pearson method.
Percentages, based on the non-missing data, will be presented with one decimal.

All listings will be presented in appendix 16.2 and sorted by site number, subject number, except in specific cases.

All statistical analyses will be described by dose cohort and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA).

Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Two interim analyses will be performed on patients with consent signed between the December 5th 2016 and the September 17th 2018 included. The first interim analysis will be performed after completion of the 28 days active study period (or have died or have been lost to follow-up) and the second after completion of 3 months follow-up period (or have died or have been lost to follow-up).
Two other interim analyses will be performed on all patients enrolled in the study. The third interim analysis will be performed after completion of the 28 days active study period (or have died or have been lost to follow-up) and the fourth after completion of 3 months follow-up period (or have died or have been lost to follow-up.

For interim analyses, cut-off date corresponds at the date of last visit for each period of interim analysis. If the last visit of the considering period is not performed, then the theoretical date will be used. The cut-off date will different according to the patient.
Management of data selection according to the cut-off date will be made as follow:

Data obtained after the cut-off will not be displayed in any listings or used for summary statistics, e.g. laboratory values of samples taken after data cut-off, AE with onset date after data cut-off, etc. will not be included in any analysis or listing.
For the following data, the following special rules apply for the derivation of the variables:

- Date of discontinuation after date of cut-off: Date and reason of discontinuation will be set to missing.
- Death later than date of cut-off: Date of death will be set to missing
- Last date known to be alive later than date of cut-off will be replaced by date of cut-off
- For adverse event occurred before or the day of cut-off, no information will be removed or modified
- For concomitant medication started before or the day of cut-off, no information will be removed or modified

Final analysis will be performed after completion of 1 year follow-up period (or have died or have been lost to follow-up) for all enrolled patients of the study.

For SMC meeting, the members will be provided with reports which will include data on recruitment, safety assessment data, and any other data as required by the SMC Members.

4.2 Sample size calculation

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile.
In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population.
Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

The 3 first patients infused (cohort 1a): 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (+/- 2 days); at least 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.
2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage.
The next 3 patients' cohort will receive a lower dose of HepaStem and the next 6 patient's cohorts (high dose cohort) will receive the higher dose.
Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Since it is a safety study, any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.
The total sample size consideration remained unchanged with a total of 12 patients

4.3 Population Analysis

4.3.1 Full analysis set (FAS)

The FAS population will include all patients screened in the study. This population will be analysed only for the disposition table. A patient will be considered as screen-failure if the eligibility has not been approved by the medical monitor or the patient did not meet the inclusion/exclusion criteria.

4.3.2 Safety population (SAF)

The safety population will include all patients entered into the study who received at least one dose of IMP. This population will be the primary dataset for all safety and efficacy analyses.

4.3.3 Per-Protocol (PP) population

The per-protocol (PP) population will include patients from the safety population without any major protocol deviation. This population will be another dataset for all efficacy analyses.

4.4 Protocol deviations

All potential deviations in relation to the study protocol and inclusion criteria of patients will initially be defined in details and searched within the database before the pre-analysis data review meeting. Results of these deviations will be reviewed on a patient-by-patient basis at the Data Review Meeting

Cohort 1a:

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old
2. Informed Consent.

N.B: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by Liver histology or Clinical and imaging examination (may include fibroscan).
4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions :

ACLF grade 1 eligible subset:

- liver failure plus cerebral and/or kidney dysfunction
- renal failure plus cerebral dysfunction
- cerebral failure plus kidney dysfunction
- coagulation failure plus cerebral and/or kidney dysfunction

Or

ACLF grade 2 eligible subset:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfunctions or failures are defined according to CLIF-C OF score as below

Diagnostic criteria of kidney and cerebral dysfunction
kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL.
cerebral: moderate impairment of brain function as defined by grade I-II HE based on West Haven criteria.
Diagnostic criteria of organ failures
liver: serum bilirubin ≥ 12 mg/dL;
kidney: serum creatinine ≥ 2 mg/dL;
cerebral: grade III-IV HE based on West Haven criteria;
coagulation: international normalized ratio [INR] ≥ 2.5

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Known prothrombotic disease or medical history of thrombotic events.
3. Gastrointestinal haemorrhage requiring blood transfusion unless controlled for more than 48h.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
5. Clinical evidence of aspergilus infection.
6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criterion.
7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion.
9. MELD score > 35.
10. Previous organ transplantation and/or ongoing immunosuppressive treatments.
11. Postoperative-decompensation following hepatectomy.
12. Renal failure due to chronic kidney disease.
13. Clinically significant left-right cardiac shunt.
14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
18. Participation to any other interventional study within the last 4 weeks.
19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

Cohort 1b, 2a, 2b:

- Inclusion criteria :

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.

2. Informed Consent.

N.B: In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

- liver histology or

- clinical and imaging examination (may include fibroscan).

4. Patient with Acute Decompensation of cirrhosis

5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)

6. The INR measurement has to be : $1.2 \leq INR < 2$

- Exclusion criteria

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.

2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.

3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion.

4. Variceal banding or sclerosis within 4 weeks before the infusion

5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.

6. Clinical evidence of Aspergilus infection.

7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion.

8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.

9. Coagulation disorders defined as :

- $INR \geq 2$

- Fibrinogen < 100 mg/dL

- Platelets < 50.000/mm3

10. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator.

11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.

12. MELD score > 30.

13. Previous organ transplantation and/or ongoing immunosuppressive treatments.

14. Postoperative-decompensation following hepatectomy.

15. Renal failure due to chronic kidney disease.

16. Clinically significant left-right cardiac shunt.

17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.

18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.

19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.

20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.

21. Participation to any other interventional study within the last 4 weeks.

22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

All decision concerning a potential withdrawal of a subject from a population of analysis due to protocol deviations will be discussed with the sponsor before data base lock. If necessary, the last version of this statistical analysis plan will be updated accordingly.

4.5 Handling of missing values

For the start dates of Adverse Event, missing dates won't be replaced, however following rules will be applied for the classification of Adverse Events as emergent/non-emergent:

- if start day is missing, and start month/year is inferior to first treatment infusion, then Adverse Event will be considered as non-emergent
- if start day is missing, and start month/year is superior or equals to first treatment infusion, then Adverse Event will be considered as emergent
- if start day and start month are missing, and start year is inferior to first treatment infusion, then Adverse Event will be considered as non-emergent
- if start day and start month are missing, and start year is superior or equals to first treatment infusion, then Adverse Event will be considered as emergent
- if start day, month and year are missing, then Adverse Event will be considered as emergent

Other missing data are not replaced and, in generally, not considered in the calculation of the percent.

4.6 Derivate variables

Age (years): (First administration date – Date of birth)

BMI (kg/m²): weight (kg) / height (m)²

Duration of inclusions (months): (Last patient included date - First patient included date)/30.44

Duration of study (months): (Last patient out date - First patient included date)/30.44

Time since X diagnosis (months): (First administration date – Date of diagnosis)/30.44

Serious Adverse Event (SAE):
The SAEs are the adverse events with the item "Serious Adverse Event" ticked "Yes". If the item is missing, the Adverse Event will be considered as Serious.

AEs related to the IMP:
The AEs related to the IMP are the AEs with the item "Relation to IMP" ticked "Definite Related", "Probable Related", "Possible Related" or missing or unknown.

AEs related to the specific study procedure:
The AEs related to the specific study procedure are the AEs with the item "Relation to specific study procedure" ticked "Definite Related", "Probable Related", "Possible Related" or missing or unknown.

AE leading to discontinuation
The AEs leading to discontinuation are the AEs with the item "Action taken regarding study drug" ticked: "drug interrupted" or" drug withdrawn"

AE leading to death:
The AE leading to death are the AEs with the item Outcome" ticked "Fatal"

AE of Special Interest (AESI) :
The Serious Adverse Event of Special Interest are defined as:
- AEs with fatal outcome
- Liver Transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

Change (absolute) at each time points: Value at each time points (first value after administration should be considered, recheck will be used only for specific case to justify) – Baseline value (value just before administration recheck included if applicable)

CLIF organ failure score system (CLIF-OF score):

CLIF-Organ Failure score system.			
Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL)
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine≥3·5 mg/dL, renal replacement
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	 >300 >357	 ≤300 - > 200 >214- ≤357	 ≤200 ≤214

For respiratory score :

- If FiO2 unit corresponds to %, used FiO2 value /100 for score calculation
- If blood gas parameters are not available then pulse oximetric saturation parameter will be used.

Statistical analysis will be based on derivate variables

CLIF-C OF score:

Diagnostic criteria of organ failures

Liver: serum bilirubin > 12 mg/dL;

Kidney: serum creatinine ≥ 2 mg/dL;

Cerebral: grade III-IV HE based on West Haven criteria;

coagulation: international normalized ratio [INR] ≥ 2.5

Use of vasopressors for circulatory failure

Respiratory failure with PaO2/FiO2 ≤200 or SpO2/FiO2 ≤214

ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF/ ACLF grade 0	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from ≥1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

Grade will be calculated using CLIF-OF derivate variables.
Statistical analysis will be based on derivate variable

West Haven Criteria of Altered mental statins in Hepatic Encephalopahy

Stage	Consciousness	Intellect and Behavior	Neurologic Findings
0	Normal	Normal	Normal examination; impaired psychomotor testing
1	Mild lack of awareness	Shortened attention span; impaired addition or subtraction	Mild asterixis or tremor
2	Lethargic	Disoriented; inappropriate behavior	Muscular rigidity and clonus; Hyperreflexia
3	Somnolent but arousable	Gross disorientation; bizarre behaviour	Muscular rigidity and clonus; Hyperreflexia
4	Coma	Coma	Decerebrate posturing

CLIF-C ACLF SCORE

Not applicable for ACLF grade 0
CLIF-C ACLF = 10 × [(0,33 × CLIF OF + 0.04 × Age{years} + 0,63 × Ln(WBC{10^9 cells/L}) – 2]
Derivate variables will be used for ACLF grade and CLIF-OF score
Statistical analysis will be based on derivate variable

CLIFconsortium acute decompensation score (CLIF-C AD) : Only applicable for ACLF grade 0
CLIF-C AD = 10 × [(0,03 × Age {years} + 0,66 × Ln(Creatinine{mg/dL} + 1.71 × Ln(INR) + 0,88 × Ln(WBC{10^9 cells/L}) – 0,05 × Sodium {mmol/L} + 8]
Derivate variable will be used for ACLF grade .

For patients of cohort 1a, CLIF-C AD score was not available in eCRF and will be calculated if necessary.
Statistical analysis will be based on derivate variable.

'Initial' or orginal MELD score:
MELD score is calculated using serum bilirubin in mg/dL, serum creatinine in mg/dL, and International Normalized Ratio (INR) and is given by the formula:
Initial MELD (i) = (0.957 * ln(Serum Creatinine) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10

Management of lower and upper values:

- if serum bilirubin < 1 then serum bilirubin is replaced by 1 for score calculation
- if serum creatinine < 1 then serum creatinine is replaced by 1 for score calculation
- if INR < 1 then INR is replaced by 1 for score calculation
- for patient with hemodialysis, serum creatinine is replaced by 4 for score calculation

'New' MELD Score (2016)
MELD Score (2016) is calculated with same information used in Initial MELD score with Na in mmol/L in addition.
New MELD Score (2016) = Initial MELD (i) + 1.32*(137-Na) – [0.033*Initial MELD (i)*(137-Na)]

Management of lower and upper values:

- if Na <125 then Na, is replaced by 125 for score calculation
- if Na >137 then Na, is replaced by 137 for score calculation
- if MELD(i) < =11 then MELD Score (2016) = MELD(i)

Statistical analysis will be based on derivate variable MELD Score (2016)

Child–Pugh:

SCORE Measure	1 point	**2 points**	**3 points**
Total bilirubin, μmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8

Prothrombin time prolongation (s) Or INR	<4.0 <1.7	4.0–6.0 1.7-2.3	> 6.0 >2.3
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Statistical analysis will be based only on investigator variable.

Child–Pugh class:
Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	**Class**	**One year survival**	**Two year survival**
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

Classes will be defined according to derivate variable of Child-Pugh calculated with INR parameter

Criteria for study treatment discontinuation:
Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration are the following:

Transitory discontinuation:

- Coagulation disorders considered as significant (INR ≥ 2, Fibrinogen < 100 mg/dL, or Platelets < 50.000/mm^3) by the PI prior to each infusion should preclude the administration of Hepastem.
- Absence of portal vein flow prior to the infusion should preclude the administration of Hepastem.
- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.
- Infusions may be restarted after recovery. If planned infusions are not performed within treatment period (+/-2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

Study withdrawal criteria:

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

5 Statistical analyses

5.1 Demographic Data

5.1.1 Patient disposition

Main study dates will be presented: first patient screened, first patient included, last patient included, last patient discontinuation. Duration of inclusions and duration of study will be calculated.

The overall number of screen-failure and the overall number of patients included in FAS population and by protocol, SAF population and in PP population will be presented.

The list of patients excluded from these populations (with reason of exclusion) will be given.

The list of patients of the FAS with informations on eligibility criteria will be provided.

The list of patients with information on non-inclusion and non-treatment reasons will be presented

The disposition of patient by country and center will be presented.

The number of patients who discontinued the study prematurely will be summarized in the SAF population. Primary reason for premature discontinuation will also be summarized.
The list of patients who discontinued the study prematurely will also be given with the reason for end of study.
A patient is considered as a patient who discontinued the study prematurely is the reason of end of study is not « end of study according to the protocol ».

The number of patients presenting at least one protocol deviation will be given. Protocol deviations as defined during the data review meeting will be listed.

5.1.1.1 Demographic and Baseline characteristics

Patients' demographic (gender, age) will be summarised by treatment dose and overall.

Descriptive statistics for the following baseline characteristics will be presented:

- Time since ACLF diagnosis (months) for patient included on protocol 1
- Time since the diagnosis of Acute decompensation of Chronic Liver Disease for patient included on protocol 3 only
- Time since admission in current hospital (days)
- Time since admission in current department (days)
- ACLF diagnosed at the time of admission in current hospitalization (Yes/No)
- ACLF diagnosed at the time of admission in current department (Yes/No)

- ACLF grade at the time of diagnosis for patient included on protocol 3 or higher only[1] (Grade 0/Grade 1/Grade 2/Grade 3)
- CLIF-C AD score for patient with ACLF grade 0 at screening (>= 60 /]45-60[/ <=45)

The cause of admission in hospital and in current department will be listed.

The following medical history (other than ACLF) will be described:
- cirrhosis history : time since cirrhosis diagnosis (months), etiology of the cirrhosis (alcoholic liver disease, hepatitis C, hepatitis B, non-alcoholic steatohepatitis, genetic disorders, autoimmune disease, other, unknown)
- cardiac, pulmonary, renal, circulatory, coagulation, metabolic/diabetes, neurology, gastrointestinal, oncology/immunology, bleeding and other history : proportion of patient with at least one overall/previous/concomitant medical history, time since diagnosis by categories, Number of pathology / patient by categories

The specification of the genetic disorders, autoimmune disease and other etiology of the cirrhosis will be listed.

The ACLF medical history will be described in term of:
- Previous episode of Acute Decompensation (Yes/No) for patient included on amendment 3 only
- Time since last episode (months) for patient included on amendment 3 only
- Previous episode of ACLF (Yes/No)
- Time since last episode (months)
- Factors(s) triggering to the current episode of AD/ACLF: Active alcoholism (Yes/No), Infection (Yes/No), Hepatitis (Yes/No), other (Yes/No), Unknown (Yes/No).

Concomitant treatment will be described by drug class and drug name.

The vital signs (height, weight, BMI), the biological analysis (see list below) only at screening will be described in this part:
- Lipase (values + abnormal CS/abnormal NCS/normal)
- Virology status: HBV antigen HBs , HCV antibodies total, HEV antibodies IgG, HEV antibodies IgM, HIV antibodies, Aspergillus (Negative/Positive/Indeterminate/Not done). The values of the positive results will be summarized.
- Pregnancy (Negative/Positive/Not applicable/Not done)
- Urine analysis : Sediment, Creatinin, Glucose, Protein, Albumin (Negative/Positive/Not done)
- Coagulation parameters: aPTT(sec), INR, Protein C, Protein S, anti-thrombin III: at screening.

ECG, culture or other fluid culture, transjugular liver biopsy at screening will be described in terms of:
- Results (Normal/Abnormal)

The specification of the abnormality of the results of Imaging-Radiology-ECG analysis will be listed.

[1] ACLF grade at the time of diagnosis was not collected in the study for the first patients.

All statistical analyses will be based on the safety and per protocol populations and described by dose cohort and overall.

5.2 Efficacy data

5.2.1 Clinical efficacy

Disease scores (West-Haven HE, CLIF-OF score (and CLIF-OF sub-scores), CLIF-C ACLF score, ACLF grade, CLIF-C AD score (Pre-ACLF patients with Acute Decompensation) MELD scores and Child Pugh score and corresponding changes from baseline/screening will be summarized by dose cohort and overall at each specified time point.

The number and the percentage of patients by ACLF grade will be described at each specified time point.

CLIF-C AD score by class (>= 60 /]45-60[/ <=45) at each specified time point will be provided.

The number and the percentage of patients having at least one organ (liver, kidney, cerebral, coagulations) failure will be described at each specified time point.

Graphical representation will be done to represent the individual raw data over time and the individual change from baseline/screening over time. A graphic with the mean change from baseline (by treatment group) and its 95% confidence interval will also be provided. A graphic with evolution of individual raw data for both CLIF-C score with different symbols used for scores (CLIF-C ACLF and CLIF-C AD) will also be provided.

All statistical analyses will be based on the safety and per protocol populations.

5.2.2 Biological efficacy

Bilirubin, creatinine, INR, albumin, sodium and Thrombin Generation Test peak values with their corresponding changes from baseline/screening will be summarized by treatment dose and overall at each specified time point.
Graphical representation will be done to represent the individual raw data over time and the individual change from baseline over time. A graphic with the mean change from baseline (by treatment group) and its 95% confidence interval will also be provided.

All statistical analyses will be based on the safety and per protocol populations.

5.3 Safety data

5.3.1 Adverse events

The number and the percentage of patients having at least the following event and the number of events will be presented at day 28:

- one AE.
- severe AE.
- one SAE.
- one AE/severe AE or SAE related to IMP.

- one AE/severe AE or SAE related to study procedure.
- one AE/severe AE or SAE having led to discontinuation of treatment.
- one SAE having led to death.
- one AE/severe AE or SAE related to IMP having led to discontinuation of treatment.
- one AE/severe AE or SAE related to study procedure having led to discontinuation of treatment.one SAE related to IMP having led to death.
- one SAE related to study procedure having led to death.

The number and the percentage of patients having at least the following event (SAE, AESI) will be presented between day 28 and month 3, and between month 3 and month 12.

- one SAE, only for analysis between day 28 and month 3
- one SAE having led to death
- Liver transplantation
- Outcome of transplantation
- Malignancies
- Hospitalisation for ACLF
- One AE possibly related to IMP

The number and percentage of patients presenting at least one AE will be summarized in a frequency table by body system organ class (SOC) and preferred terms (PT) as per MedDRA dictionary. The number of events will be also summarized in the same table. The table will be displayed by descending order based on the number of patients. Each patient will be counted only once within each classification (SOC / PT).

This analysis will be performed

- by SOC, PT at Day28 for all AE, between day 28 and month 3 only for AESI, between month 3 and month 12 only for AESI.
- by SOC, PT, and severity at Day28 for all AE, between day 28 and month 3 only for AESI, between month 3 and month 12 only for AESI

All AEs will be listed separately including at least the following items:

- Dose cohort (1a/1b/2a/2b)
- Patient number
- Age / Sex
- Infusion dates
- The preferred term (PT) and the system organ class (SOC)
- The verbatim
- Date of onset and date of end (study day) or ongoing
- Duration of the AE in days
- Intensity

- Outcome
- Relationship to studied pathology
- Relationship to IMP
- Relationship to specific study procedure
- Actions taken regarding the IMP
- Action taken regarding the event
- Evaluation of Seriousness (SAE onset date , seriousness)

Adverse events related to IMP, serious adverse events, adverse events leading to discontinuation, adverse events leading to death, adverse events of special interest will be listed separately.

5.3.2 Laboratory data

The values of the parameters and the corresponding changes from baseline/screening will be summarized by dose cohort and overall at each specified time point.

The status of the results (normal/abnormal not CS / abnormal CS) will be summarized by dose cohort and overall at each specified time point and for each parameters. Shift tables presenting the status at baseline/screening versus the worst status during study will be presented by treatment dose and overall.

The following parameters will be described:

- Common clinical laboratory tests[1]: Days 1, 4, 8, 12, 14,, 21, 28, Months 2, 3, 6 and 12.
    - White Blood Cell count, hemoglobin, hematocrit, platelets [On infusion day prior and post infusion at 4h, 24h,48h, and 72h], neutrophils, lymphocytes, monocytes, eosinophils, basophils.
    - GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT (in sec)
    - Serum albumin, sodium, potassium,
    - Blood gas : PaO2, SpO2 and FiO2

- Coagulation factors[1] prior and post infusion at 4h, 8h, 12h, 24h, 48h and 72h (Day 1 for cohort 1 / Day 1 & Day 8 for cohort 2).

[1] For first patients some parameters were not collected in the study

  - INR
  - aPTT (in sec)
  - fibrinogen
  - D-Dimers

- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X) : at screening, before each infusion and 24h post infusion.

- Thomboelastogram (central measurements only): Days 1 (before infusion and 4h post infusion),4,8(before infusion and 4h post infusion in cohort2b), 12, 14, 21, 28(blood testing in central lab) :
  - Clotting time,
  - Clot Formation Time,
  - Amplitude 10 min,
  - Maximum Clot Firmness
  - Maximum of Lysis.

- Thrombin generation test (central measurements): Days 1 (before infusion and 4h post infusion),4,8(before infusion and 4h post infusion in cohort2b), 12, 14, 21, 28(blood testing in central lab) :
  - Lag time
  - ETP
  - Peak
  - Time to peak
  - Velocity index

- Anti-HLA antibodies (class I and class II by luminex method):Screening, Day 28, Month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: Screening, Days 1,8 ,14 and 28, (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at screening, days 1, 4, 8, 12, 14 ,and 28 and Month 12

- Chest X Ray, Cardiac US Doppler, at screening and M 12:
  For Cardiac US Doppler only: If abnormal, describe (left-right shunt/Right-left Shunt/No shunt), Pulmonary arterial pressure.

At Minima, for each patient, parameters results, status, and specification of abnormalities will be listed.
“Patient fasting ? (yes/no) and Samples according to study manual (yes/no) ?” will be described for central laboratory measurement in listing only.
The values of laboratory parameters will be summarized by standard unit as defined.
The standard unit and conversion parameter are described in annex 1.

5.3.3 Physical examination

The status of the results (normal /abnormal) of Physical examination (cardiac, pulmonary, abdomen, upper extremities, lower extremities, neurologic, skin, other) will be summarized by treatment dose and overall and at each specified time point. Additionally, shift tables presenting the status at baseline/screening versus the worst status during study will be presented by treatment dose and overall.

Physical examination will be described at screening, Days 1 (before during and after infusion), 4, 8, 12, 14,, 21, 28, Months 2, 3, 6 and 12.

At minima, for each patient, parameters results, status, and specification of abnormalities will be listed.

5.3.4 Vital signs

The values of the parameters and the corresponding changes from baseline/screening will be summarized by treatment dose and overall at each specified time point.

Vital signs will be described at screening, days 1 (before during and after infusion), 4, 8, 12, 14, 21, 28, Months 2, 3, 6 and 12.

At minima, for each patient, parameters results, status, and specification of abnormalities will be listed.

The following parameters will be described: Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation

5.3.5 IMP administration

The following information will be described by treatment group and overall:

- Proportion of patient who completed all planned infusions
- Number of patients who performed each infusion for patients with an administration scheme with repeated infusions
- Duration of IMP exposure: expressed in days and calculated as

  (Date of last IMP infusion) - (date of first IMP infusion) +1

  - Total dosage of HepaStem For each infusion
  - Time from Hydrocortisone administration (minutes)
  - Total theoretical dose of HepaStem
  - Total effective dose (actually infused) of HepaStem
  - Effective (actual) dose of HepaStem infused per kg.

- Intravenous Access (Peripheral/Central)
- Duration of infusion (min)
- Minimum Flow rate during infusion (mL/min)
- Maximum Flow rate during infusion (mL/min)
- Interruption of the flow rate of HepaStem (Yes/No)
- Actual volume of Hepastem infused (mL)
- Infused Volume ratio

5.4 Analysis of sub-groups/complementary analysis

All analysis will be performed by treatment dose and overall.

6 Tables, Listings and Graphs (TLG)

STATISTICAL ANALYSIS PLAN

HEP101 – PROMETHERA BIOSCIENCES

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Final version 2.0
20 February 2019

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TABLE OF CONTENTS

1 List of abbreviations and definition of terms

Abbreviations	Definitions
ACLF	Acute on Chronic Liver Failure
AD	Acute Decompensation
AE	Adverse Event
AESI	Adverse Event of Specific Interest
aPTT	Activated Partial Thromboplastin Time
BUN	Blood Urea Nitrogen
CLIF-C	CLIF Consortium
CRP	C-Reactive Protein
CS	Clinically Significant
ECG	Electrocardiogram
ETP	Endogenous Thrombin Potential
FAS	Full analysis set
FiO2	Fraction of Inspired Oxygen
GOT	Glutamic Oxaloacetic Transaminase
GPP	Good Pharmacoepidemiology Practices
GPT	Glutamate Pyruvate Transaminase
γGT	γ Glutamyl Transferase
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HE	Hepatic Encephalopathy
HEV	Hepatitis E virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
KBP	Keyrus Biopharma
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
MSC	Mesenchymal Stem Cell
Na	Sodium
NCS	Not Clinically Significant
OF	Organ Failure
PaO2	Partial Pressure of Oxygen in Arterial Blood
PP	Per protocol population
PT	Preferred Terms (MedDRA dictionary)
SAE	Serious Adverse Event
SAF	Safety population
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SMC	Safety monitoring committee

SpO2	Pulse Oximeter Oxygen Saturation
SOC	System Organ Class (MedDRA dictionary)
TEG	Thromboelastogram
TLG	Tables, Graphs and Listings
WHO	World Health Organization

2 Introduction

This Statistical Analysis Plan (SAP) determines the frame of statistical analysis of this study in agreement with the protocol version 1.0 for Belgium dated on March 23nd 2016, the protocol version 2.0 for Belgium dated on December 13th 2016, the protocol version 3.2 dated May 11th 2017 for Belgium and version 4 dated February 15th 2018 in Bulgaria and Spain.

The SAP is reviewed, approved, and signed by the Biostatistician and Sponsor prior to database lock, at the latest.

This document will be the main reference document as far as statistical analyses are concerned.

The purpose of this document is to describe:

- The study features as per protocol in terms of objectives, study design and study conduct
- The endpoints, the study cohorts, the study variables and the derived data
- The planned statistical analysis and methodologies.

3 Study description

3.1 Study objective

3.1.1 Primary objectives

To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

3.1.2 Secondary objectives

- Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion.

- Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3.2 Study design

This is an interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts with 12 hospitalized patients in total.

5 patients were screened on the previous version of the protocol, 3 of them received HepaStem infusions. The dose of HepaStem infused for each patient is detailed below:

Patient ID	Mode of administration	Number of infusion	Dose of HepaStem per infusion	Total dose of HepaStem infused
51-01-3001	Intravenous	1	250.10^6 cells	250.10^6 cells
51-01-3002	Intravenous	2	250.10^6 cells	500.10^6 cells
51-01-3003	Intravenous	1	250.10^6 cells	250.10^6 cells

The next three patients in cohort 1 (cohort 1b) received a lower dose (minimum ten times lower) in a single infusion ($0.25.10^6$ cells /kg bodyweight with a maximum of 25.10^6 cells per infusion).
3 patients in cohort 2 (cohort 2a received twice the dose of the patients in cohort 1b ($0.5.10^6$ cells/kg bodyweight with a maximum of 50.10^6 cells per infusion)).

3 patients in cohort 2 (cohort 2b) received up to 2 doses of $0.5.10^6$ cells/ kg bodyweight 1 week apart ($0.5.10^6$ cells/kg bodyweight per infusion with a maximum of 50.10^6 cells per infusion).

The study will recruit patients who are hospitalized for Acute Decompensation of cirrhosis or ACLF and/or who develop ACLF during hospitalisation.
The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

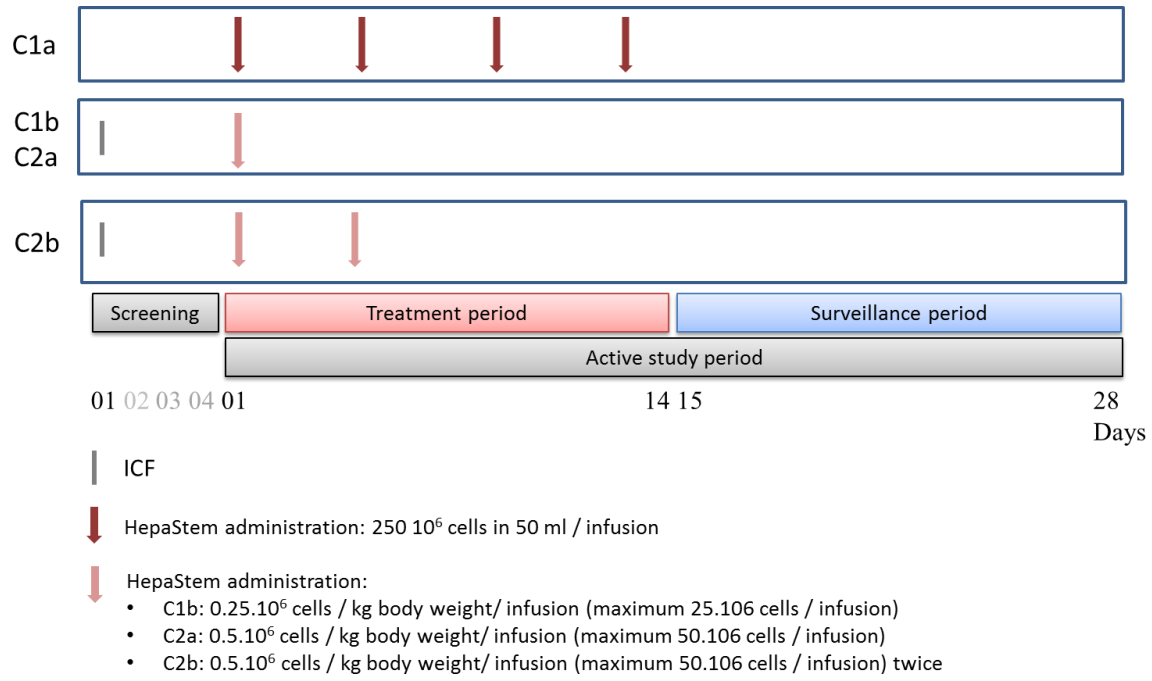
Screening period: Once informed consent is signed, may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period will last 28 days (± 2 days). The duration of the screening period plus the active period will last up to 35 days (±2 days).

Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.

Various dose regimens of HepaStem will be given, which differ in the amount of cells per infusion as shown in Figure 1.

Figure1 -Study scheme of active study period



- Planned schedule:

For cohort 1a, patients were planned to receive HepaStem on 4 infusion days spread over a 2-week period (+/- 2 days); at least a 2-day interval without infusion must be respected between infusion days.
In cohort 1a, 250 million cells in 50 ml were administrated on each infusion day, leading to a total of 1 billion cells if the patient would receive all doses. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. An infusion was expected to last 50 minutes. A single bolus of 100 mg hydrocortisone would be given 15 to 30 min before each HepaStem infusion.

- Actual schedule:

In cohort 1a, 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (+/- 2 days); at least a 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem

suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.

- Planned schedule based on protocol version 4:

For cohort 1b: 3 patients will receive HepaStem in a single infusion ($0.25.10^6$ cells per kg body weight with a maximum of 25.10^6 cells). One infusion of maximum 5 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of HepaStem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion

For cohort 2a: 3 patients will receive HepaStem in a single infusion ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells). One infusion of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of HepaStem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion.

For cohort 2b: 3 patients will receive HepaStem in 2 repeated infusions one week apart ($0.5.10^6$ cells per kg body weight with a maximum of 50.10^6 cells per infusion). 2 infusions of maximum 10 ml reconstituted HepaStem suspension will be given. In case the patient's body weight is below 100 kg, the exact volume of HepaStem will be adapted accordingly. A single bolus of 100 mg hydrocortisone will be given 15 to 30 min before HepaStem infusion. The parameters of the patients will be checked before the second infusion administration (see Criteria for study treatment discontinuation)

Patients are treated in a stepwise approach under the continuous supervision of a Safety Monitoring Committee (SMC), composed of external members and Promethera members only for open session:

As a minimum, the safety data will be reviewed by the SMC at the following time points:

- For each cohort (1b, 2a and 2b), when the first evaluable patient has received HepaStem infusion (complete scheme or premature stop), the safety data will be reviewed by the SMC. The SMC will then advise on the continuation of the enrolment and on the dosage and frequency of administration for the next patients.
- When 3 evaluable patients of each cohort have received HepaStem infusions (complete scheme or premature stop), the SMC will then advise on the enrolment of patient in the next cohort.

Long-term safety follow-up: After completion of the active study period, patients will be followed-up up to 1 year post first HepaStem infusion in the long-term safety follow-up period.

After completion of this study, patients will be followed-up in the Patient Registry.

3.3 Study plan

During the screening and treatment period, patients will be hospitalised in intermediate or ICUs or standard units, depending of the severity of the patient disease.
Once informed consent is signed, the screening period may last from 1 to 4 days for protocol version 1, 2, 3.1 and 3.2 and up to 7 days for protocol version 4, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history will be recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients will be treated in a stepwise approach.

During the treatment period, a study visit will be performed on Days 1, 4, 8, 12 and 14 (± 2 days for each visit form the Day 4 visit) including the evaluation listed in table 2.

On HepaStem infusion days before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, TEG (if already performed as a part of the clinical routine and up to investigator's judgment), a liver echography and Doppler, and evaluation of disease scorings will be performed, as listed below. These assessments will be performed on each planned infusion day even if HepaStem infusions are prematurely stopped.

The careful evaluation of these parameters will allow or not the infusion or treatment discontinuation.

On the other days during the hospital stay, patients will be followed-up according to usual practice.

After the treatment period, study visits will be done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients will enter the - follow-up period up to 1 year, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to the 28 days visit, all SAEs will be collected. After the 28 days visit, up to Month 12 visit, safety will be based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem.
At the Month 12 study visit, patients will be invited to be included in the long term safety follow up study (5- years).

Table 2 Study Flowchart

Period	**Screening Period**	**Active period**							*Long term follow-up*			
	Baseline	**Treatment Period**					**Surveillance Period**					
Time	**Maximum 7 days prior D1**	Infusion D1	D4[b]	D8[c]	D12[b]	D14[b]	D21[b]	D28[b]	M2[d]	M3[d]	M6[d]	M12[e]
Informed Consent	X											
Eligibility criteria	X X											
Demography & Medical History	X											
Physical exam	X	«	X	«	X	X	X	X	X	X	X	X
Vital Sign	X	↔	X	↔	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD (pre-ACLF patients with Acute Decompensation), MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	«%	X	«%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	«	X	«	X	X	X	X	X	X	X	X
Lipase	X											
Coagulation 1 : INR, aPTT	X	+	X	+	X	X	X	X	X	X	X	X
Coagulation 2 : C-Protein, S-Protein, Anti-Thrombin III	X§											
Virology status (HbS Ag, HCV, HEV, HIV), Aspergillosis test	X											
Coagulation 3 : Fibrinogen, D-Dimers, TEG®		+		+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	&		&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	«		«		X		X				
TEG, TG	X	*	X	*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	«	X	«	X	X	X	X				X
Chest X-Ray	©											X
Cardiac US Doppler	©	≠										X
ECG	©											
Blood culture or other fluid culture	A											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10[6] cells /kg body weight with a maximum of 25.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2a : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]										
Cohort 2b : Infusion of 0.5.10[6] cells /kg body weight with a maximum of 50.10[6] cells + Hydrocortison (100mg)		X[a]		X[a]								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a) Hydrocortisone given 15-30 min before HepaStem infusion
b) ± 2 days
c) ± 2 days with at least 7 days interval without infusion
d) ± 2 weeks
e) ± 1 month
« Before each infusion.
A: If already performed during same admission, results collected
%: On infusion day, platelets measurement to be performed prior and post infusion at 4h, 24h, 48h and 72h[1].
© if not already performed during same admission; if already performed, results collected
↔ before, during, after each infusion
≠: cardiac US to be performed after infusion
@: Optional, only if measurement can be done locally and up to investigator's judgment
+: On infusion day: prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion[2] (frequency of these exams can be increased up to the investigator's judgment.)
*: Before infusion and 4h post infusion[3]
AESI: only Adverse Event of Special Interest to be reported
TEG: Thromboelastogram, TG: Thrombin generation
&: Prior and 24h after infusion
§: In case of deterioration of the coagulation, these measurements will be repeated.

[1] Modification of time point due to an error in the protocol version 4 (prior and post infusion at 1h, 24h, 48 and 72h)
[2] Modification of time points due to errors in the protocol version 4 (prior and 1h, 3h, 5h, 8h, 12h, 18h, 24 and 72h post infusion)
[3] Modification of time point due to an error in the protocol version 4 (before and 3h post infusion)

3.4 Changes in the conduct of the study

Cf; amendment

4 Statistical methods

4.1 General statistical considerations

All statistical analyses will be performed with the SAS software version 9.4.

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed. The analysis will be limited to descriptive statistics by dose cohort.

Standard descriptive statistics will be used for quantitative and categorical variables.

Quantitative variables will be presented using the number of observed values, number of missing observations, mean, standard deviation, and median, minimum and maximum.
When required, Confidence intervals will be computed based on the Wald method.

Categorical variables will be presented using counts and percentages of patients. The number of missing observations will also be presented.
When required, Confidence intervals will be computed based on the Clopper-Pearson method.
Percentages, based on the non-missing data, will be presented with one decimal.

All listings will be presented in appendix 16.2 and sorted by site number, subject number, except in specific cases.

All statistical analyses will be described by dose cohort and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA).

Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Two interim analyses will be performed on patients with consent signed between the December 5th 2016 and the September 17th 2018 included. First interim analysis will be performed after completion of the 28 days active study period (or have died or have been lost to follow-up) and the second after completion of 3 months follow-up period (or have died or have been lost to follow-up).
Two other interim analyses will be performed on all patients enrolled in the study. The third interim analysis will be performed after completion of the 28 days active study period (or have died or have been lost to follow-up) and the fourth after completion of 3 months follow-up period (or have died or have been lost to follow-up.

For interim analyses, cut-off date corresponds at the date of last visit for each period of interim analysis. If the last visit of the considering period is not performed, then the theoretical date will be used. The cut-off date will different according to the patient.
Management of data selection according to the cut-off date will be made as follow:

Data obtained after the cut-off will not be displayed in any listings or used for summary statistics, e.g. laboratory values of samples taken after data cut-off, AE with onset date after data cut-off, etc. will not be included in any analysis or listing.
For the following data, the following special rules apply for the derivation of the variables:

- Date of discontinuation after date of cut-off: Date and reason of discontinuation will be set to missing.
- Death later than date of cut-off: Date of death will be set to missing
- Last date known to be alive later than date of cut-off will be replaced by date of cut-off
- For adverse event occurred before or the day of cut-off, no information will be removed or modified
- For concomitant medication started before or the day of cut-off, no information will be removed or modified

Final analysis will be performed after completion of 1 year follow-up period (or have died or have been lost to follow-up) for all enrolled patients in the study.

For SMC meeting, the members will be provided with reports which will include data on recruitment, safety assessment data, and any other data as required by the SMC Members.

4.2 Sample size calculation

The published clinical experience with Mesenchymal Stem Cell (MSC) (with known procoagulant properties) in various clinical conditions is supporting the safety of MSCs administered through IV route. HepaStem has been administered at variable doses in 20 paediatric patients through the portal vein under anticoagulation medication, with an acceptable safety profile.
In the present study, HepaStem will be administered for the first time through peripheral IV route to ACLF cirrhotic patients without concomitant anti-coagulation. This justifies an open study in a small number of patients closely monitored to investigate the safety and explore efficacy parameters of HepaStem in this population.
Starting with a dose regimen close to those reported as being safe in MSC clinical trials in a cohort of 6 patients, then a higher dosage in a second cohort of 6 patients that appears to be an acceptable approach in ACLF patients for whom no specific therapeutic or curative treatment exist.

The 3 first patients infused (cohort 1a): 3 patients received HepaStem (250.10^6 cells per infusion) on 1 or 2 infusion days spread over a 2-week period (+/- 2 days); at least 2-day interval without infusion was respected between infusion days. On each infusion day, one infusion of 50 ml reconstituted HepaStem suspension was given. A single bolus of 100 mg hydrocortisone was given 15 to 30 min before each HepaStem infusion.
2 of these 3 patients experienced severe bleeding which led to a decrease of the dosage.
The next 3 patients' cohort will receive a lower dose of HepaStem and the next 6 patient's cohorts (high dose cohort) will receive the higher dose.
Enrolled patients not meeting the inclusion/exclusion criteria will be considered as screening failure and will be replaced. Since it is a safety study, any patients receiving at least one HepaStem infusion will be considered as an included and evaluable patient.
The total sample size consideration remained unchanged with a total of 12 patients.

4.3 Population Analysis

4.3.1 Full analysis set (FAS)

The FAS population will include all patients screened in the study. This population will be analysed only for the disposition table. A patient will be considered as screen-failure if the eligibility has not been approved by the medical monitor or the patient did not meet the inclusion/exclusion criteria.

4.3.2 Safety population (SAF)

The safety population will include all patients entered into the study who received at least one dose of IMP. This population will be the primary dataset for all safety and efficacy analyses.

4.3.3 Per-Protocol (PP) population

The per-protocol (PP) population will include patients from the safety population without any major protocol deviation. This population will be another dataset for all efficacy analyses.

4.4 Protocol deviations

All potential deviations in relation to the study protocol and inclusion criteria of patients will initially be defined in details and searched within the database before the pre-analysis data review meeting. Results of these deviations will be reviewed on a patient-by-patient basis at the Data Review Meeting.

Cohort 1a:

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old
2. Informed Consent.

N.B: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by Liver histology or Clinical and imaging examination (may include fibroscan).
4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions:

ACLF grade 1 eligible subset:

- liver failure plus cerebral and/or kidney dysfunction
- renal failure plus cerebral dysfunction
- cerebral failure plus kidney dysfunction
- coagulation failure plus cerebral and/or kidney dysfunction

Or

ACLF grade 2 eligible subset:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfunctions or failures are defined according to CLIF-C OF score as below

Diagnostic criteria of kidney and cerebral dysfunction
kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL.
cerebral: moderate impairment of brain function as defined by grade I-II HE based on West Haven criteria.
Diagnostic criteria of organ failures
liver: serum bilirubin ≥ 12 mg/dL;
kidney: serum creatinine ≥ 2 mg/dL;
cerebral: grade III-IV HE based on West Haven criteria;
coagulation: international normalized ratio [INR] ≥ 2.5

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Known prothrombotic disease or medical history of thrombotic events.
3. Gastrointestinal haemorrhage requiring blood transfusion unless controlled for more than 48h.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
5. Clinical evidence of *Aspergilus* infection.
6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criterion.
7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion.
9. MELD score > 35.
10. Previous organ transplantation and/or ongoing immunosuppressive treatments.
11. Postoperative-decompensation following hepatectomy.
12. Renal failure due to chronic kidney disease.
13. Clinically significant left-right cardiac shunt.
14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
18. Participation to any other interventional study within the last 4 weeks.
19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

Cohort 1b, 2a, 2b:

- Inclusion criteria:

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.

2. Informed Consent.

N.B: In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

- liver histology or

- clinical and imaging examination (may include fibroscan).

4. Patient with Acute Decompensation of cirrhosis

5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)

6. The INR measurement has to be: $1.2 \leq INR < 2$

- Exclusion criteria

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.

2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.

3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion.

4. Variceal banding or sclerosis within 4 weeks before the infusion

5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.

6. Clinical evidence of *Aspergilus* infection.

7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion.

8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.

9. Coagulation disorders defined as:

- INR ≥ 2

- Fibrinogen < 100 mg/dL

- Platelets < 50.000/mm^3

10. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator.

11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.

12. MELD score > 30.

13. Previous organ transplantation and/or ongoing immunosuppressive treatments.

14. Postoperative-decompensation following hepatectomy.

15. Renal failure due to chronic kidney disease.

16. Clinically significant left-right cardiac shunt.

17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.

18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.

19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.

20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.

21. Participation to any other interventional study within the last 4 weeks.

22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

All decision concerning a potential withdrawal of a subject from a population of analysis due to protocol deviations will be discussed with the sponsor before data base lock. If necessary, the last version of this statistical analysis plan will be updated accordingly.

4.5 Handling of missing values

For the start dates of Adverse Event, missing dates won't be replaced, however following rules will be applied for the classification of Adverse Events as emergent/non-emergent:

- if start day is missing, and start month/year is inferior to first treatment infusion, then Adverse Event will be considered as non-emergent
- if start day is missing, and start month/year is superior or equals to first treatment infusion, then Adverse Event will be considered as emergent
- if start day and start month are missing, and start year is inferior to first treatment infusion, then Adverse Event will be considered as non-emergent
- if start day and start month are missing, and start year is superior or equals to first treatment infusion, then Adverse Event will be considered as emergent
- if start day, month and year are missing, then Adverse Event will be considered as emergent

Other missing data are not replaced and, in generally, not considered in the calculation of the percent.

4.6 Derivate variables

Age (years): (First administration date – Date of birth)

BMI (kg/m²): weight (kg) / height (m)²

Duration of inclusions (months): (Last patient included date - First patient included date)/30.44

Duration of study (months): (Last patient out date - First patient included date)/30.44

Time since X diagnosis (months): (First administration date – Date of diagnosis)/30.44

Serious Adverse Event (SAE):
The SAEs are the adverse events with the item "Serious Adverse Event" ticked "Yes". If the item is missing, the Adverse Event will be considered as Serious.

AEs related to the IMP:
The AEs related to the IMP are the AEs with the item "Relation to IMP" ticked "Definite Related", "Probable Related", "Possible Related" or missing or unknown.

AEs related to the specific study procedure:
The AEs related to the specific study procedure are the AEs with the item "Relation to specific study procedure" ticked "Definite Related", "Probable Related", "Possible Related" or missing or unknown.

AE leading to discontinuation
The AEs leading to discontinuation are the AEs with the item "Action taken regarding study drug" ticked: "drug interrupted" or" drug withdrawn"

AE leading to death:
The AE leading to death are the AEs with the item Outcome" ticked "Fatal"

AE of Special Interest (AESI):
AESI are collected after D28 visit and correspond to AE with one of the following items ticked:
- AEs with fatal outcome
- Liver Transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

Liver transplantation:
AE liver transplantation corresponds to AE with "Liver transplant" as PT name.

Thrombin Generation Test: Negative values will be substituted by 0.

Baseline:
Last non missing value before first IMP intake (except for height).

Change (absolute) at each time points: Value at each time points (first value after administration should be considered, recheck will be used only for specific case to justify) – Baseline value (value just before administration recheck included if applicable)

CLIF organ failure score system (CLIF-OF score):

CLIF-Organ Failure score system.

Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine ≥3·5 mg/dL, renal replacement
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	 >300 >357	 ≤300 - > 200 >214- ≤357	 ≤200 ≤214

For respiratory score:

- If FiO2 unit corresponds to %, used FiO2 value /100 for score calculation
- If blood gas parameters are not available then pulse oximetric saturation parameter will be used.

Statistical analysis will be based on derivate variables

CLIF-C OF score:

Diagnostic criteria of organ failures

Liver: serum bilirubin > 12 mg/dL;

Kidney: serum creatinine ≥ 2 mg/dL;

Cerebral: grade III-IV HE based on West Haven criteria;

coagulation: international normalized ratio [INR] ≥ 2.5

Use of vasopressors for circulatory failure

Respiratory failure with PaO2/FiO2 ≤200 or SpO2/FiO2 ≤214

ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF/ ACLF grade 0	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from ≥1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

Grade will be calculated using CLIF-OF derivate variables.
Statistical analysis will be based on derivate variable

West Haven Criteria of Altered mental statins in Hepatic Encephalopathy

Stage	Consciousness	Intellect and Behavior	Neurologic Findings
0	Normal	Normal	Normal examination; impaired psychomotor testing
1	Mild lack of awareness	Shortened attention span; impaired addition or subtraction	Mild asterixis or tremor
2	Lethargic	Disoriented; inappropriate behavior	Muscular rigidity and clonus; Hyperreflexia
3	Somnolent but arousable	Gross disorientation; bizarre behaviour	Muscular rigidity and clonus; Hyperreflexia
4	Coma	Coma	Decerebrate posturing

CLIF-C ACLF SCORE
Not applicable for ACLF grade 0
CLIF-C ACLF = 10 × [(0.33 × CLIF OF + 0.04 × Age{years} + 0.63 × Ln(WBC{10^9 cells/L}) – 2]
Derivate variables will be used for ACLF grade and CLIF-OF score
Statistical analysis will be based on derivate variable

CLIF consortium acute decompensation score (CLIF-C AD): Only applicable for ACLF grade 0
CLIF-C AD = 10 × [(0.03 × Age {years} + 0.66 × Ln(Creatinine{mg/dL} + 1.71 × Ln(INR) + 0.88 × Ln(WBC{10^9 cells/L}) – 0.05 × Sodium {mmol/L} + 8]
Derivate variable will be used for ACLF grade.

For patients of cohort 1a, CLIF-C AD score was not available in eCRF and will be calculated if necessary.
Statistical analysis will be based on derivate variable.

'Initial' or original MELD score:
MELD score is calculated using serum bilirubin in mg/dL, serum creatinine in mg/dL, and International Normalized Ratio (INR) and is given by the formula:
Initial MELD (i) = (0.957 * ln(Serum Creatinine) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10

Management of lower and upper values:

- if serum bilirubin < 1 then serum bilirubin is replaced by 1 for score calculation
- if serum creatinine < 1 then serum creatinine is replaced by 1 for score calculation
- if INR < 1 then INR is replaced by 1 for score calculation
- for patient with hemodialysis, serum creatinine is replaced by 4 for score calculation

'New' MELD Score (2016)
MELD Score (2016) is calculated with same information used in Initial MELD score with Na in mmol/L in addition.
New MELD Score (2016) = Initial MELD (i) + 1.32*(137-Na) – [0.033*Initial MELD (i)*(137-Na)]

Management of lower and upper values:

- if Na <125 then Na, is replaced by 125 for score calculation
- if Na >137 then Na, is replaced by 137 for score calculation
- if MELD(i) < =11 then MELD Score (2016) = MELD(i)

Statistical analysis will be based on derivate variable MELD Score (2016)

Child–Pugh:

SCORE Measure	1 point	**2 points**	**3 points**
Total bilirubin, μmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin (g/dL)	>3.5	2.8–3.5	<2.8
Prothrombin time prolongation (s) Or INR	<4.0 <1.7	4.0–6.0 1.7-2.3	> 6.0 >2.3
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Statistical analysis will be based only on investigator variable.

Child–Pugh class:
Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	**Class**	**One year survival**	**Two year survival**
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

Classes will be defined according to derivate variable of Child-Pugh calculated with INR parameter

Criteria for study treatment discontinuation:
Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration are the following:

Transitory discontinuation:

- Coagulation disorders considered as significant (INR ≥ 2, Fibrinogen < 100 mg/dL, or Platelets < 50.000/mm^3) by the PI prior to each infusion should preclude the administration of HepaStem.
- Absence of portal vein flow prior to the infusion should preclude the administration of HepaStem.
- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.
- Infusions may be restarted after recovery. If planned infusions are not performed within treatment period (+/-2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.

- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

Study withdrawal criteria:

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

5 Statistical analyses

5.1 Demographic Data

5.1.1 Patient disposition

Main study dates will be presented: first patient screened, first patient included, last patient included, last patient discontinuation. Duration of inclusions and duration of study will be calculated.

The overall number of screen-failure and the overall number of patients included in FAS population and by protocol, SAF population and in PP population will be presented.

The list of patients excluded from these populations (with reason of exclusion) will be given.

The list of patients of the FAS with information on eligibility criteria will be provided.

The list of patients with information on non-inclusion and non-treatment reasons will be presented.

The disposition of patient by country and center will be presented.

The number of patients who discontinued the study prematurely will be summarized in the SAF population. Primary reason for premature discontinuation will also be summarized.
The list of patients who discontinued the study prematurely will also be given with the reason for end of study.
A patient of the SAF is considered as a patient who discontinued the study prematurely if the reason of end of study is not « end of study according to the protocol ».

The number of patients presenting at least one protocol deviation will be given. Protocol deviations as defined during the data review meeting will be listed.

5.1.1.1 Demographic and Baseline characteristics

Patients' demographic (gender, age) will be summarised by treatment dose and overall.
Descriptive statistics for the following baseline characteristics will be presented:

- Time since ACLF diagnosis (months) for patient included on protocol 1
- Time since the diagnosis of Acute decompensation of Chronic Liver Disease for patient included on protocol 3 only
- Time since admission in current hospital (days)
- Time since admission in current department (days)
- ACLF diagnosed at the time of admission in current hospitalization (Yes/No)
- ACLF diagnosed at the time of admission in current department (Yes/No)
- ACLF grade at the time of diagnosis for patient included on protocol 3 or higher only[1] (Grade 0/Grade 1/Grade 2/Grade 3)

[1] ACLF grade at the time of diagnosis was not collected in the study for the first patients.

The cause of admission in hospital and in current department will be listed.

The following medical history (other than ACLF) will be described:

- cirrhosis history: time since cirrhosis diagnosis (months), etiology of the cirrhosis (alcoholic liver disease, hepatitis C, hepatitis B, non-alcoholic steatohepatitis, genetic disorders, autoimmune disease, other, unknown)
- cardiac, pulmonary, renal, circulatory, coagulation, metabolic/diabetes, neurology, gastrointestinal, oncology/immunology, bleeding and other history: proportion of patient with at least one overall/previous/concomitant medical history, time since diagnosis by categories, Number of pathology / patient by categories

The specification of the genetic disorders, autoimmune disease and other etiology of the cirrhosis will be listed.

The ACLF medical history will be described in term of:

- Previous episode of Acute Decompensation (Yes/No) for patient included on amendment 3 only
- Time since last episode (months) for patient included on amendment 3 only
- Previous episode of ACLF (Yes/No)
- Time since last episode (months)
- Factors(s) triggering to the current episode of AD/ACLF: Active alcoholism (Yes/No), Infection (Yes/No), Hepatitis (Yes/No), other (Yes/No), Unknown (Yes/No).

Concomitant treatment will be described by drug class and drug name.

The vital signs (height, weight, BMI), the biological analysis (see list below) only at screening will be described in this part:

- Lipase (values and by classes abnormal CS/abnormal NCS/normal)
- Virology status: HBV antigen HBs, HCV antibodies total, HEV antibodies IgG, HEV antibodies IgM, HIV antibodies, Aspergillus (Negative/Positive/Indeterminate/Not done). The values of the positive results will be summarized.
- Pregnancy (Negative/Positive/Not applicable/Not done)
- Urine analysis: Sediment, Creatinin, Glucose, Protein, Albumin (Negative/Positive/Not done)
- Coagulation parameters (values and by classes abnormal CS/abnormal NCS/normal): aPTT(sec), INR, Protein C, Protein S, anti-thrombin III: at screening.

ECG, culture or other fluid culture, transjugular liver biopsy at screening will be described in terms of:

- Results (Normal/Abnormal)

The specification of the abnormality of the results of Imaging-Radiology-ECG analysis will be listed.

All statistical analyses will be based on the safety and per protocol populations and described by dose cohort and overall.

5.2 Efficacy data

5.2.1 Clinical efficacy

All analyses will be provided by treatment dose and overall in the SAF and PP populations.
The occurrence of liver transplantations will be specified for all tables (footnote) and graphics (symbol or footnote).

Disease scores will be described in terms of:

- West-Haven criteria of HE: values at baseline and each post baseline time point and shift table presenting the grade at baseline versus the worst grade during study will be presented.
- CLIF-OF score and sub-scores with their components: values at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.
- Organ failure (Yes/No) based on CLIF-C OF score: The number and the percentage of patients at baseline and each post-baseline time points will be presented by organ (liver, kidney, cerebral, coagulation, circulatory and respiratory).
- ACLF grade: the number and the percentage of patients at baseline and each post-baseline time points will be presented.
- CLIF-C ACLF score: values at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.
- CLIF-C AD score (Pre-ACLF patients with Acute Decompensation): values (quantitative and by class: ≥60 /]45-60[/ ≤45) at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.
- MELD scores and number of dialysis in the past week (<2 / ≥2): values at baseline and each post baseline time point and change from baseline at each post baseline time point (not applicable for class description) will be described.

Child Pugh score: values (quantitative and by class: A / B / C) at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.

Graphical representation will be done to represent the individual raw data over time and the individual change from baseline to post-baseline values. A graphic with the mean +/- standard deviation of change from baseline to post-baseline values (by treatment group) will also be provided.
A graphic with evolution of individual raw data for both CLIF-C score with different symbols used for scores (CLIF-C ACLF and CLIF-C AD) will also be provided.

5.2.2 Biological efficacy

All analyses will be provided by treatment dose and overall in the SAF and PP populations.
The occurrence of liver transplantations will be specified for all tables (footnote) and graphics (symbol or footnote).

Bilirubin, creatinine, INR, albumin, sodium and Thrombin Generation Test peak values with their corresponding changes from baseline to post-baseline values will be summarized at baseline and each post-baseline time point.
Graphical representation will be done to represent the individual raw data over time and the individual change from baseline to post-baseline values. A graphic with the mean +/- standard deviation of change from baseline to post-baseline values (by treatment group) will also be provided.

5.3 Safety data

5.3.1 Adverse events

All analyses will be provided by treatment dose and overall in the SAF population.
The occurrence of liver transplantations will be specified for all tables (footnote).

The number and the percentage of patients having at least the following event and the number of events will be presented at day 28:

- one AE.
- severe AE.
- one SAE.
- one AE/severe AE or SAE related to IMP.
- one AE/severe AE or SAE related to study procedure.
- one AE/severe AE or SAE having led to discontinuation of treatment.
- one SAE having led to death.
- one AE/severe AE or SAE related to IMP having led to discontinuation of treatment.
- one AE/severe AE or SAE related to study procedure having led to discontinuation of treatment.
- one SAE related to IMP having led to death.
- one SAE related to study procedure having led to death.

The number and the percentage of patients having at least the following event (SAE, AESI) will be presented between day 28 and month 3, and between month 3 and month 12.

- one SAE, only for analysis between day 28 and month 3
- one SAE having led to death
- Liver transplantation
- Outcome of transplantation
- Malignancies
- Hospitalisation for ACLF

- One AE possibly related to IMP

The number and percentage of patients presenting at least one AE will be summarized in a frequency table by body system organ class (SOC) and preferred terms (PT) as per MedDRA dictionary. The number of events will be also summarized in the same table. The table will be displayed by descending order based on the number of patients. Each patient will be counted only once within each classification (SOC / PT).

This analysis will be performed

- by SOC, PT at Day28 for all AE, between day 28 and month 3 only for AESI, between month 3 and month 12 only for AESI.
- by SOC, PT, and severity at Day28 for all AE, between day 28 and month 3only for AESI, between month 3 and month 12 only for AESI

All AEs will be listed separately including at least the following items:

- Dose cohort (1a/1b/2a/2b)
- Patient number
- Age / Sex
- Infusion dates
- The preferred term (PT) and the system organ class (SOC)
- The verbatim
- Date of onset and date of end (study day) or ongoing
- Duration of the AE in days
- Intensity
- Outcome
- Relationship to studied pathology
- Relationship to IMP
- Relationship to specific study procedure
- Actions taken regarding the IMP
- Action taken regarding the event
- Evaluation of Seriousness (SAE onset date, seriousness)

Adverse events related to IMP, serious adverse events, adverse events leading to discontinuation, adverse events leading to death, adverse events of special interest will be listed separately.

5.3.2 Laboratory data

All analyses will be provided in the safety population.
The occurrence of liver transplantations will be specified for all tables (footnote).

The values of the parameters and the corresponding changes from baseline to post-baseline values will be summarized by dose cohort and overall at baseline and each post-baseline time point.

The status of the results (normal/abnormal not CS / abnormal CS) will be summarized by dose cohort and overall at baseline and each post-baseline time point and for each parameter. Shift tables presenting the status at baseline versus the worst status during study will be presented by treatment dose and overall.

The following parameters will be described:

- Common clinical laboratory tests[1] at baseline, days 4, 8, 12, 14, 21, 28, and months 2, 3, 6 and 12.
    - White Blood Cell count, hemoglobin, hematocrit, neutrophils, lymphocytes, monocytes, eosinophils, basophils.
    - platelets (additional measures on infusion day: post infusion at 4h, 24h, 48h, and 72h at day 1, and day 8 in cohort 2b),
    - GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT (in sec)
    - Serum albumin, sodium, potassium,
    - Blood gas: PaO2, SpO2 and FiO2

- Coagulation factors[1]: at baseline, and day 8 and additional measures on infusion day (post infusion at 4h, 8h, 12h, 24h, 48h and 72h at day 1, and day 8 in cohort 2b).
    - INR
    - aPTT (in sec)
    - fibrinogen
    - D-Dimers

- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X): at baseline and day 8 and additional measures on infusion day (24h post infusion at day 1, and day 8 in cohort 2b).

- Thomboelastogram (central measurements only): at baseline and days 4, 8 12, 14, 21 and 28, and additional measures on infusion day (4h post infusion at day 1, and day 8 in cohort 2b) (blood testing in central lab):
    - Clotting time

[1] For first patients some parameters were not collected in the study

  - Clot Formation Time
  - Amplitude 10 min
  - Maximum Clot Firmness
  - Maximum of Lysis

- Thrombin generation test (central measurements): at baseline and days 4, 8 12, 14, 21 and 28, and additional measures on infusion day (4h post infusion at day 1, and day 8 in cohort 2b) (blood testing in central lab):
  - Lag time
  - ETP
  - Peak
  - Time to peak
  - Velocity index

- Anti-HLA antibodies (class I and class II by luminex method): at baseline, day 28, and month 3, 6 and 12 (blood testing in central lab)
- Inflammatory and anti-inflammatory cytokines: at baseline and days 8, 14 and 28 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at baseline, days 4, 8, 12, 14 and 28 and month 12

- Chest X Ray and Cardiac US Doppler: at baseline and month 12:
  For Cardiac US Doppler only: If abnormal, describe (left-right shunt/Right-left Shunt/No shunt), Pulmonary arterial pressure.

At Minima, for each patient, parameters results, status, and specification of abnormalities will be listed.
"Patient fasting ? (yes/no) and Samples according to study manual (yes/no) ?" will be described for central laboratory measurement in listing only.
The values of laboratory parameters will be summarized by standard unit as defined in SDTM database.

5.3.3 Physical examination

All analyses will be provided by treatment dose and overall in the safety population.
The occurrence of liver transplantations will be specified for all tables (footnote).

The status of the results (normal /abnormal) of Physical examination (cardiac, pulmonary, abdomen, upper extremities, lower extremities, neurologic, skin, other) will be summarized by treatment dose and overall and baseline and at each post-baseline time point. Additionally, shift tables presenting the status at baseline versus the worst status during study will be presented by treatment dose and overall.

Physical examination will be described at baseline, days 4, 8, 12, 14, 21 and 28 and months 2, 3, 6 and 12.

At minima, for each patient, parameters results, status, and specification of abnormalities will be listed.

5.3.4 Vital signs

The values of the parameters and the corresponding changes from baseline to post-baseline values will be summarized by treatment dose and overall at baseline and each post-baseline time point in the safety population.
The occurrence of liver transplantations will be specified for all tables (footnote).

Vital signs will be described at baseline, days 1, 4, 8, 12, 14, 21 and 28 and months 2, 3, 6 and 12, and additional measurements on infusion day (during and after infusion at day 1, and day 8 in cohort 2b).

At minima, for each patient, parameters results, status, and specification of abnormalities will be listed.

The following parameters will be described: Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation.

5.3.5 IMP administration

The following information will be described by treatment group and overall in the safety population:

- Proportion of patient who completed all planned infusions
- Number of patients who performed each infusion for patients with an administration scheme with repeated infusions
- Duration of IMP exposure: expressed in days and calculated as

  (Date of last IMP infusion) - (date of first IMP infusion) +1
- Total dosage of HepaStem
- For each infusion:
  - Time from Hydrocortisone administration (minutes)
  - Total theoretical dose of HepaStem
  - Total effective dose (actually infused) of HepaStem
  - Effective (actual) dose of HepaStem infused per kg.
  - Intravenous Access (Peripheral/Central)
  - Duration of infusion (min)
  - Minimum Flow rate during infusion (mL/min)

- Maximum Flow rate during infusion (mL/min)
- Interruption of the flow rate of HepaStem (Yes/No)
- Actual volume of HepaStem infused (mL)
- Infused Volume ratio

The occurrence of liver transplantations will be specified for all tables (footnote).

5.4 Analysis of sub-groups/complementary analysis

All analysis will be performed by treatment dose and overall.

6 Tables, Listings and Graphs (TLG)

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STATISTICAL ANALYSIS PLAN

HEP101 – PROMETHERA BIOSCIENCES

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Final version 3.0
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TABLE OF CONTENTS

1 List of abbreviations and definition of terms

Abbreviations	Definitions
ACLF	Acute on Chronic Liver Failure
AD	Acute Decompensation
AE	Adverse Event
AESI	Adverse Event of Specific Interest
aPTT	Activated Partial Thromboplastin Time
BUN	Blood Urea Nitrogen
CLIF-C	CLIF Consortium
CRP	C-Reactive Protein
CS	Clinically Significant
ECG	Electrocardiogram
ETP	Endogenous Thrombin Potential
FAS	Full analysis set
FiO2	Fraction of Inspired Oxygen
GOT	Glutamic Oxaloacetic Transaminase
GPP	Good Pharmacoepidemiology Practices
GPT	Glutamate Pyruvate Transaminase
γGT	γ Glutamyl Transferase
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HE	Hepatic Encephalopathy
HEV	Hepatitis E virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
KBP	Keyrus Biopharma
LLOD	Lower Limit Of Detection
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
MSC	Mesenchymal Stem Cell
Na	Sodium
NCS	Not Clinically Significant
OF	Organ Failure
PaO2	Partial Pressure of Oxygen in Arterial Blood
PP	Per protocol population
PT	Preferred Terms (MedDRA dictionary)
SAE	Serious Adverse Event
SAF	Safety population
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDTM	Study Data Tabulation Model

SMC	Safety monitoring committee
SpO2	Pulse Oximeter Oxygen Saturation
SOC	System Organ Class (MedDRA dictionary)
TEG	Thromboelastogram
TLG	Tables, Graphs and Listings
WHO	World Health Organization

2 Introduction

This Statistical Analysis Plan (SAP) determines the frame of statistical analysis of this study in agreement with the protocol version 1.0 for Belgium dated on March 23nd 2016, the protocol version 2.0 for Belgium dated on December 13th 2016, the protocol version 3.2 dated May 11th 2017 for Belgium, the protocol version 4.0 dated on February 15th 2018 in Bulgaria and Spain, the protocol version 5.0 dated on June 26th 2018 in Belgium, the protocol version 5.1 dated on June 26th 2018 in France and the protocol version 6.0 dated on December 14th 2018 in Belgium and the protocol version 6.1 dated on February 05th, 2019 in France.

The SAP is reviewed, approved, and signed by the Biostatistician and Sponsor prior to database lock, at the latest.

This document will be the main reference document as far as statistical analyses are concerned.

The purpose of this document is to describe:

- The study features as per protocol in terms of objectives, study design and study conduct
- The endpoints, the study cohorts, the study variables and the derived data
- The planned statistical analysis and methodologies.

3 Study description

3.1 Study objective

3.1.1 Primary objectives

To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

3.1.2 Secondary objectives

- Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion.

- Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3.2 Study design

This is an interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts.

Initially, according to protocol version 1.0 and 2.0, patients with CLIF-ACLF grade 1 or grade 2 were to be included. Patients in the first cohort (6 patients) were planned to receive 4 HepaStem infusions over 2 weeks at a dose of 250 x 10^6 cells per infusion (approximatively 3.5 x 10^6 cells/kg). Patients in the second cohort (6 patients) were planned to receive a double dose with the same schedule. However, in the first cohort (referred as cohort 1a), 2 bleeding events were assessed as SAEs causally related to HepaStem by the Investigator. The bleeding was successfully managed, and both patients recovered.
Following 2 SAEs of severe bleeding observed in 2 ACLF patients with pre-existing coagulation disturbances (1 of the same type in each patient) which led to a safety signal, changes were included in protocol version 2 in order to increase the safety of the study (1) the inclusion criteria were modified to allow inclusion of less severely-ill patients for the next 3 patients; 2) the dose was reduced (proposed 5x reduction of dose/infusion) for the next 3 patients, with an increase in dose for the next cohorts, (3) implementation of additional safety measures with regular blood test for coagulation status before, during and after infusion for up to 24 hrs or longer if medically indicated, (4) implementation of stepwise patient inclusion, the data of the next 3 patients to be reviewed by the SMC.
Three less severely ill patients (ACLF grade 0) received a single infusion of 0.25 x 10^6 cells/kg (cohort 1b). Following consultation of the SMC members, enrolment into the next dose cohort could begin: 3 patients received single infusion of 0.5 x 10^6 cells/kg (cohort 2a), and 3 patients received 2 infusion of 0.5 x 10^6 cells/kg (cohort 2b).
Following consultation of the SMC members, inclusion of more severely ill patients was allowed, as well as the dose increase for additional cohorts 2c and 2d (single or double infusion of 1.0 x 10^6 cells/kg per infusion). Four more patients received 2 infusion of 0.5 x 10^6 cells/kg (cohort 2b), 3 patients received a single infusion of 1.0 x 10^6 cells/kg (cohort 2c) and 5 patients received two infusions of 1.0 x 10^6 cells/kg per infusion (cohort 2d).

The recruitment is completed (24 patients in total), and the Safety follow-up is ongoing, with an anticipated LSLV in July 2020.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

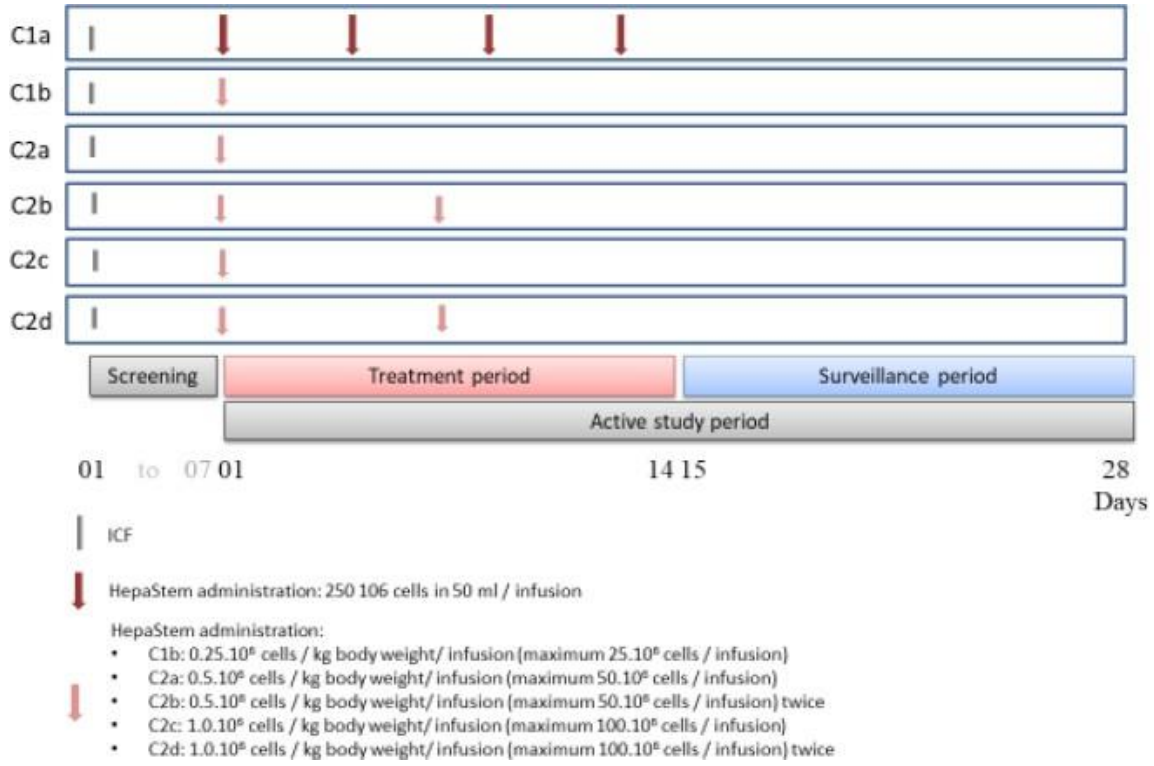
Screening period: Once informed consent is signed, may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period lasts 28 days (± 2 days). The duration of the screening period plus the active period lasts up to 35 days (±2 days).

Enrolled patients not meeting the inclusion/exclusion criteria are considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion is considered as an included and evaluable patient.

Various dose regimens of HepaStem have been given, which differ in the amount of cells per infusion as shown in Figure 1.

Figure1 -Study scheme of active study period



Long-term safety follow-up: After completion of the active study period, patients are followed-up up to 1 year post first HepaStem infusion in the safety follow-up period.

After completion of this study, patients will be invited to participate in a long-term safety follow-up study for 5 years (PROLONGSTEM).

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3.3 Study plan

During the screening and treatment period, patients are hospitalised in intermediate or ICUs or standard units, depending of the severity of the patient disease.
Once informed consent is signed, the screening period may last from 1 to 4 days for protocol version 1, 2, 3.1 and 3.2 and up to 7 days for protocol versions 4.0, 5.0, 5.1, 6.0 & 6.1, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history is recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria are reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients are treated in a stepwise approach.

During the treatment period, a study visit is performed on Days 1, 4, 8, 12 and 14 (± 2 days for each visit form the Day 4 visit) including the evaluation listed in table 2.

On HepaStem infusion days before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, coagulation factors (from protocol version 3.0), TEG (if already performed as a part of the clinical routine and up to investigator's judgment), a liver echography and Doppler, and evaluation of disease scorings is performed, as listed below. These assessments are performed on each planned infusion day even if HepaStem infusions are prematurely stopped.

The careful evaluation of these parameters allows or not the infusion or treatment discontinuation. Vital signs evaluation is also performed during and after infusion and, from the protocol version 3.0, coagulation parameters and TEG are closely monitored after infusion as listed in study flowchart.

On the other days during the hospital stay, patients are followed-up according to usual practice.

After the treatment period, study visits are done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients enter the follow-up period up to 1 year, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to the 28 days visit, all SAEs are collected. After the 28 days visit, up to Month 12 visit, safety is based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem.
At the Month 12 study visit, patients will be invited to be included in the long-term safety follow up study (5- years).

Table 2 Study Flowchart

Period	Screening Period	Active period							*Long term follow-up*			
	Baseline	**Treatment Period**					**Surveillance Period**					
Time	**Over 1-7 days prior D1**	Infusion D1	D4 ± 2 days	D8 ± 2 days	D12 ± 2 days	D14 ± 2 days	**D21** ± 2 days	**D28** ± 2 days	**M2** ± 2 weeks	**M3** ± 2 weeks	**M6** ± 2 weeks	**M12** ± 1 month
Informed Consent	X											
Eligibility criteria	X											
Demography & Medical History	X											
Physical exam	X	Xa	X	Xa	X	X	X	X	X	X	X	X
Vital Sign	X	Xb	X	Xb	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD, MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	X%	X	X%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	X%	X	X%	X	X	X	X	X	X	X	X
Lipase & Coagulation 2: C-protein, S-protein, Anti-Thrombin III	X											
Coagulation 1 : INR, aPTT	X	X+	X	X+	X	X	X	X	X	X	X	X
Virology status (HbS Ag, HCV, HEV, HIV), Aspergillosis test	X											
Coagulation 3 : Fibrinogen	X	X+		X+								
Coagulation 3 : D-Dimers, optional local TEG		X+		X+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	Xa		Xa		X		X				
TEG, TG	X	X*	X	X*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	Xa	X	Xa	X	X	X	X				X
Chest X-Ray	©											X
ECG	©											
Cardiac US Doppler	©	≠										X
Blood culture or other fluid culture	c											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X										
Cohort 2a & b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2b only								
Cohort 2c & d : Infusion of 1.0.10⁶ cells /kg body weight with a maximum of 100.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2d only								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a: On infusion day: before infusion
b: On infusion day: before, during and after infusion
C: Only if performed during the same admission
%: On infusion day: all parameters are measured prior infusion /platelets measurement to be performed prior and post infusion at 4h, 24h, 48h and 72h.
+: On infusion day: prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion
&: On infusion day: prior and 24h after infusion
©: if not already performed during same admission. If already performed, results collected
*: On infusion day: prior and 4h after infusion
≠: cardiac US to be performed after infusion
AESI: only Adverse Event of Special Interest to be reported

3.4 Changes in the conduct of the study

Cf; amendment

4 Statistical methods

4.1 General statistical considerations

All statistical analyses will be performed with the SAS software version 9.4.

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed.

Standard descriptive statistics will be used for quantitative and categorical variables.

Quantitative variables will be presented using the number of observed values, number of missing observations, mean, standard deviation, and median, minimum and maximum.
When required, Confidence intervals will be computed based on the Wald method.

Evolution between baseline and post-baseline visits will be estimated using the non-parametric approach of Hodges & Lehmann for paired sample. Estimate and its two-sided 95% confidence interval of the difference between visits will be provided.

Comparison between groups will be estimated using the non-parametric approach of Hodges & Lehmann for independent samples. Estimate and its two-sided 95% confidence interval of the difference between groups will be provided.

Categorical variables will be presented using counts and percentages of patients. The number of missing observations will also be presented.
When required, Confidence intervals will be computed based on the Clopper-Pearson method.
Percentages, based on the non-missing data, will be presented with one decimal.

All listings will be presented in appendix 16.2 and sorted by site number, subject number, except in specific cases.

All statistical analyses, except comparison, will be described by planned dose cohort and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA).

Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Two interim analyses will be performed on patients with consent signed between the December 5th 2016 and the September 17th 2018 included. First interim analysis will be performed after completion of the 28 days active study period (or have died or have been lost to follow-up) and the second after completion of 3 months follow-up period (or have died or have been lost to follow-up).
Two other interim analyses will be performed on all patients enrolled in the study. The third interim analysis will be performed after completion of the 28 days active study period (or have died or have been lost to follow-up) and the fourth after completion of 3 months follow-up period (or have died or have been lost to follow-up.

For interim analyses, cut-off date corresponds at the date of last visit for each period of interim analysis. If the last visit of the considering period is not performed, then the theoretical date will be used. The cut-off date will different according to the patient.
Management of data selection according to the cut-off date will be made as follow:

Data obtained after the cut-off will not be displayed in any listings or used for summary statistics, e.g. laboratory values of samples taken after data cut-off, AE with onset date after data cut-off, etc. will not be included in any analysis or listing.
For the following data, the following special rules apply for the derivation of the variables:

- Date of discontinuation after date of cut-off: Date and reason of discontinuation will be set to missing.
- Death later than date of cut-off: Date of death will be set to missing
- Last date known to be alive later than date of cut-off will be replaced by date of cut-off
- For adverse event occurred before or the day of cut-off, no information will be removed or modified
- For concomitant medication started before or the day of cut-off, no information will be removed or modified

Final analysis will be performed after completion of 1 year follow-up period (or have died or have been lost to follow-up) for all enrolled patients in the study.

For SMC meeting, the members will be provided with reports which will include data on recruitment, safety assessment data, and any other data as required by the SMC Members.

4.2 Sample size calculation

No sample size calculation was performed.
The total sample size depended on the recommendations given by SMC in order to protect patient safety based on a risk assessment.
In total, 24 evaluable patients were included.

4.3 Population Analysis

4.3.1 Full analysis set (FAS)

The FAS population will include all patients screened in the study. This population will be analysed only for the disposition table. A patient will be considered as screen-failure if the eligibility has not been approved by the medical monitor or the patient did not meet the inclusion/exclusion criteria.

4.3.2 Safety population (SAF)

The safety population will include all patients entered into the study who received at least one dose of IMP. This population will be the primary dataset for all safety and efficacy analyses.

4.3.3 Per-Protocol (PP) population

The per-protocol (PP) population will include patients from the safety population without any major protocol deviation. This population will be another dataset for all efficacy analyses.

4.4 Protocol deviations

All potential deviations in relation to the study protocol and inclusion criteria of patients will initially be defined in details and searched within the database before the pre-analysis data review meeting. Results of these deviations will be reviewed on a patient-by-patient basis at the Data Review Meeting.

Protocol version 1.0 and 2.0 (Cohort 1a):

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old
2. Informed Consent.

N.B: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by Liver histology or Clinical and imaging examination (may include fibroscan).
4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions:

ACLF grade 1 eligible subset:

- liver failure plus cerebral and/or kidney dysfunction

- renal failure plus cerebral dysfunction
- cerebral failure plus kidney dysfunction
- coagulation failure plus cerebral and/or kidney dysfunction

Or

ACLF grade 2 eligible subset:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfunctions or failures are defined according to CLIF-C OF score as below

Diagnostic criteria of kidney and cerebral dysfunction
kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL.
cerebral: moderate impairment of brain function as defined by grade I-II HE based on West Haven criteria.
Diagnostic criteria of organ failures
liver: serum bilirubin ≥ 12 mg/dL;
kidney: serum creatinine ≥ 2 mg/dL;
cerebral: grade III-IV HE based on West Haven criteria;
coagulation: international normalized ratio [INR] ≥ 2.5

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Known prothrombotic disease or medical history of thrombotic events.
3. Gastrointestinal haemorrhage requiring blood transfusion unless controlled for more than 48h.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
5. Clinical evidence of *Aspergilus* infection.
6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criterion.
7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion.
9. MELD score > 35.
10. Previous organ transplantation and/or ongoing immunosuppressive treatments.
11. Postoperative-decompensation following hepatectomy.

12. Renal failure due to chronic kidney disease.
13. Clinically significant left-right cardiac shunt.
14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
18. Participation to any other interventional study within the last 4 weeks.
19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

Protocol version 3.2 and 4.0 (Cohort 1b, 2a, 2b):

- Inclusion criteria:

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.

2. Informed Consent.

N.B: In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

- liver histology or

- clinical and imaging examination (may include fibroscan).

4. Patient with Acute Decompensation of cirrhosis

5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)

6. The INR measurement has to be: $1.2 \leq INR < 2$

- Exclusion criteria

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.

2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.

3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion.

4. Variceal banding or sclerosis within 4 weeks before the infusion

5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.

6. Clinical evidence of *Aspergilus* infection.

7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion.

8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.

9. Coagulation disorders defined as:

- INR ≥ 2

- Fibrinogen < 100 mg/dL

- Platelets < 50.000/mm^3

10. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator.

11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.

12. MELD score > 30.

13. Previous organ transplantation and/or ongoing immunosuppressive treatments.

14. Postoperative-decompensation following hepatectomy.

15. Renal failure due to chronic kidney disease.

16. Clinically significant left-right cardiac shunt.

17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.

18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.

19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.

20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.

21. Participation to any other interventional study within the last 4 weeks.

22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

All decision concerning a potential withdrawal of a subject from a population of analysis due to protocol deviations will be discussed with the sponsor before data base lock. If necessary, the last version of this statistical analysis plan will be updated accordingly.

Protocol version 5.0, 5.1, 6.0 and 6.1 (Cohort 2b, 2c, 2d):

- Inclusion criteria:

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfil all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.

2. Signed Informed Consent.

N.B: In case of hepatic encephalopathy, if the patient is not able to understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

- liver histology or

- clinical and imaging examination (may include fibroscan).

4. Patient with Acute Decompensation of cirrhosis

5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)

- Exclusion criteria

Any of the following criteria will exclude a patient from the study:

1. Thrombosis of the portal vein.

2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.

Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.

3. Ongoing uncontrolled bleeding.

4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.

5. Clinical evidence of Aspergillus infection.

6. Circulatory failure defined by inability to maintain a mean Blood pressure ≥ 70 despite use of vasopressors

7. Mechanical ventilation due to respiratory failure.

8. Coagulation disorders defined as:

- Fibrinogen < 80 mg/dL

- Platelets < 40.000/mm3

9. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator.

10. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.

11. MELD score > 35.

12. Previous organ transplantation and/or ongoing immunosuppressive treatments.

13. Postoperative-decompensation following hepatectomy.

14. Renal failure due to chronic kidney disease.

15. Clinically significant left-right cardiac shunt.

16. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.

17. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. In case of suspicion of HCC, all exam should be done to confirm or not the diagnosis prior enrolment.

18. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.

19. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.

20. Participation to any other interventional study within the last 4 weeks.

21. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

All decision concerning a potential withdrawal of a subject from a population of analysis due to protocol deviations will be discussed with the sponsor before data base lock. If necessary, the last version of this statistical analysis plan will be updated accordingly.

4.5 Handling of missing values

For the start dates of Adverse Event, missing dates won't be replaced, however following rules will be applied for the classification of Adverse Events as emergent/non-emergent (ie AE occurs during or outside the study period) :

- if start day is missing, and start month/year is inferior to first treatment infusion, then Adverse Event will be considered as non-emergent
- if start day is missing, and start month/year is superior or equals to first treatment infusion, then Adverse Event will be considered as emergent
- if start day and start month are missing, and start year is inferior to first treatment infusion, then Adverse Event will be considered as non-emergent
- if start day and start month are missing, and start year is superior or equals to first treatment infusion, then Adverse Event will be considered as emergent
- if start day, month and year are missing, then Adverse Event will be considered as emergent

For the start dates of medical history:

- if only start day is missing, then the 1rst day of the month will be taken for the imputation
- if start day and start month are missing, then the 1rst January will be taken for the imputation

Other missing data are not replaced and, in generally, not considered in the calculation of the percent.

4.6 Derivate variables

Age (years): (First consent date (by patient or legal representative) – Date of birth)

BMI (kg/m²): weight (kg) / height (m)²

Duration of inclusions (months): (Last patient included date - First patient included date)/30.44

Duration of study (months): (Last patient out date - First patient included date)/30.44

Time since X diagnosis (months): (First administration date – Date of diagnosis)/30.44

Serious Adverse Event (SAE):
The SAEs are the adverse events with the item "Serious Adverse Event" ticked "Yes". If the item is missing, the Adverse Event will be considered as Serious.

AEs related to the IMP:
The AEs related to the IMP are the AEs with the item "Relation to IMP" ticked "Definite Related", "Probable Related", "Possible Related" or missing or unknown.

AEs related to the specific study procedure:
The AEs related to the specific study procedure are the AEs with the item "Relation to specific study procedure" ticked "Definite Related", "Probable Related", "Possible Related" or missing or unknown.

AE leading to discontinuation
The AEs leading to discontinuation are the AEs with the item "Action taken regarding study drug" ticked: "drug interrupted" or" drug withdrawn"

AE leading to death:
The AE leading to death are the AEs with the item Outcome" ticked "Fatal"

AE of Special Interest (AESI):
AESI are collected after D28 visit and correspond to AE with one of the following items ticked:

- AEs with fatal outcome
- Liver Transplantation and outcome of the transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

Liver transplantation:
AE liver transplantation corresponds to AE with "Liver transplant" as PT name.

Thrombin Generation Test: Negative values will be substituted by 0.

Baseline:
Last non missing value before first IMP intake (except for height).

Change (absolute) at each time points: Value at each time points (first value after administration should be considered, recheck will be used only for specific case to justify) – Baseline value (value just before administration recheck included if applicable)

CLIF organ failure score system (CLIF-OF score):

CLIF-Organ Failure score system.

Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine ≥3·5 mg/dL, renal replacement therapy
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	 >300 >357	 ≤300 - > 200 >214- ≤357	 ≤200 ≤214

For respiratory score:

- If FiO2 unit corresponds to %, used FiO2 value /100 for score calculation
- If blood gas parameters are not available then pulse oximetric saturation parameter will be used.

Statistical analysis will be based on derivate variables

CLIF-C OF score:

Diagnostic criteria of organ failures
Liver: serum bilirubin > 12 mg/dL;
Kidney: serum creatinine ≥ 2 mg/dL;
Cerebral: grade III-IV HE based on West Haven criteria;
coagulation: international normalized ratio [INR] ≥ 2.5
Use of vasopressors for circulatory failure
Respiratory failure with PaO2/FiO2 ≤200 or SpO2/FiO2 ≤214

ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF/ ACLF grade 0	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from ≥1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

Grade will be calculated using CLIF-OF derivate variables.
Statistical analysis will be based on derivate variable

West Haven Criteria of Altered mental statins in Hepatic Encephalopathy

Stage	Consciousness	Intellect and Behavior	Neurologic Findings
0	Normal	Normal	Normal examination; impaired psychomotor testing
1	Mild lack of awareness	Shortened attention span; impaired addition or subtraction	Mild asterixis or tremor
2	Lethargic	Disoriented; inappropriate behavior	Muscular rigidity and clonus; Hyperreflexia
3	Somnolent but arousable	Gross disorientation; bizarre behaviour	Muscular rigidity and clonus; Hyperreflexia
4	Coma	Coma	Decerebrate posturing

CLIF-C ACLF SCORE
Not applicable for ACLF grade 0
CLIF-C ACLF = $10 \times [(0.33 \times \text{CLIF OF} + 0.04 \times \text{Age\{years\}} + 0.63 \times \text{Ln(WBC\{}10^9 \text{ cells/L\})} - 2]$
Derivate variables will be used for ACLF grade and CLIF-OF score
Statistical analysis will be based on derivate variable

CLIF consortium acute decompensation score (CLIF-C AD):
CLIF-C AD = $10 \times [(0.03 \times \text{Age \{years\}} + 0.66 \times \text{Ln(Creatinine\{mg/dL\}} + 1.71 \times \text{Ln(INR)} + 0.88 \times \text{Ln(WBC\{}10^9 \text{ cells/L\})} - 0.05 \times \text{Sodium \{mmol/L\}} + 8]$
Derivate variable will be used for ACLF grade.

For patients of cohort 1a, CLIF-C AD score was not available in eCRF and will be calculated.
Statistical analysis will be based on derivate variable.

'Initial' or original MELD score:
MELD score is calculated using serum bilirubin in mg/dL, serum creatinine in mg/dL, and International Normalized Ratio (INR) and is given by the formula:
Initial MELD (i) = (0.957 * ln(Serum Creatinine) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10

Management of lower and upper values:

- if serum bilirubin < 1 then serum bilirubin is replaced by 1 for score calculation
- if serum creatinine < 1 then serum creatinine is replaced by 1 for score calculation

- if INR < 1 then INR is replaced by 1 for score calculation
- for patient with hemodialysis, serum creatinine is replaced by 4 for score calculation

'New' MELD Score (2016)
MELD Score (2016) is calculated with same information used in Initial MELD score with Na in mmol/L in addition.
New MELD Score (2016) = Initial MELD (i) + 1.32*(137-Na) – [0.033*Initial MELD (i)*(137-Na)]

Management of lower and upper values:

- if Na <125 then Na, is replaced by 125 for score calculation
- if Na >137 then Na, is replaced by 137 for score calculation
- if MELD(i) < =11 then MELD Score (2016) = MELD(i)

Statistical analysis will be based on derivate variable MELD Score (2016)

Child–Pugh:

SCORE Measure	1 point	**2 points**	**3 points**
Total bilirubin, μmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin (g/dL)	>3.5	2.8–3.5	<2.8
Prothrombin time prolongation (s) Or INR	<4.0 <1.7	4.0–6.0 1.7-2.3	> 6.0 >2.3
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Statistical analysis will be based only on investigator variable.

Child–Pugh class:
Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	**Class**	**One year survival**	**Two year survival**
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

Classes will be defined according to derivate variable of Child-Pugh calculated with INR parameter

FIB-4:
FIB-4 = (age * AST) / (platelet count * SQRT(ALT))
With age in year, ALT and AST in IU/L and platelet count in 10^9/L

APRI:
APRI = ((AST / AST Upper level of normal) / Platelet count)) * 100
With AST and AST Upper level of normal in IU/L and platelet count in 10^9/L

Cytokines:

The Lower Limit of Detection (LLOD) for each cytokine is indicated in the following table:

Parameter	LLOD (pg/mL)
CRP	0,83
Eotaxin	3,26
Eotaxin-3	1,77
GM-CSF	0,16
IFN-γ	0,37
IL-1α	0,09
IL-1β	0,05
IL-2	0,09
IL-4	0,02
IL-5	0,14
IL-6	0,06
IL-7	0,12
IL-8	0,07
IL-8 (HA)	95,6
IL-10	0,04
IL-12/IL-23p40	0,33
IL-12p70	0,11
IL-15	0,15
IL-16	2,83
IL-17A	0,31
IP-10	0,37
MCP-1	0,09
MCP-4	1,69
MDC	1,22
MIP-1α	3,02
MIP-1β	0,17
TARC	0,22
TNF-α	0,04
TNF-β	0,08
VEGF-A	1,12

Results reported as "NaN" correspond, in this study, at values below fit curve limit, and they will be substituted by the LLOD/√2.
Values greater or equal to LLOD will be considered "In range", and all other values, even those substituted, will be identified as below LLOD.

Antibacterial treatment:
Antibacterial treatment will be identified by the sponsor from the Drug name.

Automated-calculated TCC/mL dose cohort:
Automated-calculated cell concentration dose cohorts will be defined by the sponsor based on a new method for the calculation of the total cell concentration and the number of infusion performed:
Cohort 1 - 1st pat.: Patients of cohort 1a
Cohort 2 - 0.6-0.8 x 1: Automated-calculated TCC/mL dose between 0.6 and 0.8 million cells / kg bodyweight and 1 infusion
Cohort 3 - 0.6 x 2: Automated-calculated TCC/mL dose of 0.6 million cells / kg bodyweight and 2 infusions
Cohort 4 – 1.2 x 1: Automated-calculated TCC/mL dose of 1.2 million cells / kg bodyweight and 1 infusion
Cohort 5 – 1.2 x 2: Automated-calculated TCC/mL dose of 1.2 million cells / kg bodyweight and 2 infusions

Naïve and non-naïve to glucocorticosteroids:
Patients naïve and non-naïve to glucocorticosteroids will be identified by the sponsor based on the previous concomitant treatment. Non-naïve patients correspond to patients receiving glucocorticosteroids before screening and others patients corresponds to naïve patients even if they were treated by glucocorticosteroids after the screening.

Criteria for study treatment discontinuation:
Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration are the following:

Transitory discontinuation:

- Coagulation disorders considered as significant (INR ≥ 2, Fibrinogen < 100 mg/dL, or Platelets < 50.000/mm^3) by the PI prior to each infusion should preclude the administration of HepaStem.
    - For protocol versions 3 and 4: INR ≥ 2, Fibrinogen < 100 mg/dL, or Platelets < 50.000/mm^3
    - For protocol versions 5 and 6: Fibrinogen < 80 mg/dL, or Platelets < 40.000/mm^3
- Absence of portal vein flow prior to the infusion should preclude the administration of HepaStem.
- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.

- Infusions may be restarted after recovery. If planned infusions are not performed within treatment period (+/-2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

Study withdrawal criteria:

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator
- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

5 Statistical analyses

5.1 Demographic Data

5.1.1 Patient disposition

Main study dates will be presented: first patient screened, first patient included, last patient included, last patient discontinuation. Duration of inclusions and duration of study will be calculated.

The overall number of screen-failure and the overall number of patients included in FAS population and by protocol, SAF population and in PP population will be presented.

The list of patients excluded from these populations (with reason of exclusion) will be given.

The list of patients of the FAS with information on eligibility criteria will be provided.

The list of patients with information on non-inclusion and non-treatment reasons will be presented.

The disposition of patient by country and center will be presented.

The number of patients who discontinued the study prematurely will be summarized in the SAF population. Primary reason for premature discontinuation will also be summarized.
The list of patients who discontinued the study prematurely will also be given with the reason for end of study.
A patient of the SAF is considered as a patient who discontinued the study prematurely if the reason of end of study is not « end of study according to the protocol ».

The number of patients presenting at least one protocol deviation will be given. Protocol deviations as defined during the data review meeting will be listed.

5.1.1.1 Demographic and Baseline characteristics

Patients' demographic (gender, age) will be summarised by planned treatment dose and overall. Descriptive statistics for the following baseline characteristics will be presented:

- Time since ACLF diagnosis (months) for patient included on protocol 1
- Time since the diagnosis of Acute decompensation of Chronic Liver Disease for patient included on protocol 3 only
- Time since admission in current hospital (days)
- Time since admission in current department (days)
- ACLF diagnosed at the time of admission in current hospitalization (Yes/No)
- ACLF diagnosed at the time of admission in current department (Yes/No)
- ACLF grade at the time of diagnosis for patient included on protocol 3 or higher only[1] (Grade 0/Grade 1/Grade 2/Grade 3)

Values at screening and at D1 prior infusion and the change from screening to D1 prior infusion for quantitative parameters or shift table for qualitative parameters will be described for the following parameters:

- ACLF grade
- Scores: West Haven criteria, New MELD score and CLIF-C AD
- Common clinical laboratory tests: WBC, neutrophils, Bilirubin and CRP
- Coagulation parameters: Platelets, INR and Fibrinogen
- Thromboelastogram (central measurements only): Clotting time, Clot Formation Time, Amplitude 10 min, Maximum Clot Firmness and Maximum of Lysis
- Thrombin generation test (central measurements): Lag time, ETP, Peak, Time to peak, Velocity index

The cause of admission in hospital and in current department will be listed.

[1] ACLF grade at the time of diagnosis was not collected in the study for the first patients.

The following medical history (other than ACLF) will be described:

- cirrhosis history: time since cirrhosis diagnosis (months), etiology of the cirrhosis (alcoholic liver disease, hepatitis C, hepatitis B, non-alcoholic steatohepatitis, genetic disorders, autoimmune disease, other, unknown)
- cardiac, pulmonary, renal, circulatory, coagulation, metabolic/diabetes, neurology, gastrointestinal, oncology/immunology, bleeding and other history: proportion of patient with at least one overall/previous/concomitant medical history, time since diagnosis by categories, Number of pathology / patient by categories
- Overall and concomitant medical history will also be described by SOC and PT.

The specification of the genetic disorders, autoimmune disease and other etiology of the cirrhosis will be listed.

The ACLF medical history will be described in term of:

- Previous episode of Acute Decompensation (Yes/No) for patient included from amendment 3
- Time since last episode of Acute Decompensation (months) for patient included from amendment 3
- Previous episode of ACLF (Yes/No)
- Time since last episode of ACLF (months)
- Factors(s) triggering to the current episode of AD/ACLF: Active alcoholism (Yes/No), Infection (Yes/No), Hepatitis (Yes/No), other (Yes/No), Unknown (Yes/No).

Concomitant treatment will be described by drug class and drug name.

Antibacterial concomitant treatment will be described by drug class and drug name too.

The vital signs (height, weight, BMI), the biological analysis (see list below) only at screening will be described in this part:

- Lipase (values and by classes abnormal CS/abnormal NCS/normal)
- Virology status: HBV antigen HBs, HCV antibodies total, HEV antibodies IgG, HEV antibodies IgM, HIV antibodies, Aspergillus (Negative/Positive/Indeterminate/Not done). The values of the positive results will be summarized.
- Pregnancy (Negative/Positive/Not applicable/Not done)
- Urine analysis: Sediment, Creatinin, Glucose, Protein, Albumin (Negative/Positive/Not done)
- Coagulation parameters (values and by classes abnormal CS/abnormal NCS/normal): aPTT(sec), INR, Protein C, Protein S, anti-thrombin III: at screening.

ECG, culture or other fluid culture, transjugular liver biopsy at screening will be described in terms of:

- Results (Normal/Abnormal)

The specification of the abnormality of the results of Imaging-Radiology-ECG analysis will be listed.

All statistical analyses will be based on the safety and per protocol populations and described by dose cohort and overall.

5.2 Efficacy data

5.2.1 Clinical efficacy

All analyses will be provided by planned treatment dose and overall in the SAF and PP populations, unless stated otherwise. The occurrence of liver transplantations will be specified for all tables (footnote) and graphics (symbol or footnote).

Disease scores will be described in terms of:

- West-Haven criteria of HE: values at baseline and each post baseline time point and shift table presenting the grade at baseline versus the worst grade during study will be presented.
- CLIF-OF score and sub-scores with their components: values at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.
- Organ failure (Yes/No) based on CLIF-C OF score: The number and the percentage of patients at baseline and each post-baseline time points will be presented by organ (liver, kidney, cerebral, coagulation, circulatory and respiratory).
- ACLF grade: the number and the percentage of patients at baseline and each post-baseline time points will be presented.
- CLIF-C ACLF score: values at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.
- CLIF-C AD score: values (quantitative and by class: ≥60 /]45-60[/ ≤45) at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.
- MELD scores and number of dialysis in the past week (<2 / ≥2): values at baseline and each post baseline time point and change from baseline at each post baseline time point (not applicable for class description) will be described.
- Child Pugh score: values (quantitative and by class: A / B / C) at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.
- FIB-4 score: values at baseline, D28, M3, M6 and M12 and change from baseline to D28, M3, M6 and M12 will be described.
- APRI scores: values at baseline, D28, M3, M6 and M12 and change from baseline to D28, M3, M6 and M12 will be described.

Graphical representations will be done to represent:

- Individual profiles (overall and by planned cohort dose) of values at baseline and at post-baseline visits and changes from baseline to post-baseline visits will be done only on the SAF population
- Mean evolution (+/- standard deviation) of change from baseline to post-baseline values (by planned treatment group) will also be provided, except for West-Haven criteria.

Evolution from baseline to D28 and M3 will be described only for the patients of the SAF population without liver transplantation at D28 (respectively M3) using paired Hodges & Lehmann estimator with its 95% confidence interval for the new MELD score and the Child-Pugh score.

All previous analyses (tables and graphics), except comparison between visits, for new MELD score and Child-Pugh score will be also performed by automated-calculated cell concentration dose cohorts on the SAF population.

5.2.2 Biological efficacy

All analyses will be provided by planned treatment dose and overall in the SAF and PP populations, unless stated otherwise. The occurrence of liver transplantations will be specified for all tables (footnote) and graphics (symbol or footnote).

Values at baseline and post-baseline visits and changes from baseline to post-baseline visits will be summarized for the following parameters:

.

- Common clinical laboratory: WBC, Neutrophil count, Bilirubin, creatinine, albumin, sodium and CRP
- Coagulation parameters, only on SAF population: platelets, INR and aPTT
- Thomboelastogram (central measurements only), only on SAF population: Clotting time and Maximum Clot Firmness
- Thrombin generation test (central measurements), only on SAF population: ETP, Peak and Velocity index

Graphical representations will be done for the previous parameters to represent:

- Individual profiles of values at baseline and at post-baseline visits (only values prior infusion will be presented) and changes from baseline to post-baseline visits (only values prior infusion will be presented) will be done only on the SAF population
- Mean evolution (+/- standard deviation) of change from baseline to post-baseline values (only values prior infusion will be presented) (by planned treatment group) will also be provided.

The same analyses (tables and graphics for individual profiles and mean evolution), but only on the SAF population, will be performed on the short term around infusion with values prior infusion, post-infusion and at the first visit after infusion for the following parameters:

- Coagulation parameters: platelets, INR, aPTT, Fibrinogen and D-dimers
- Thomboelastogram (central measurements only): Clotting time and Maximum Clot Firmness
- Thrombin generation test (central measurements): ETP, Peak and Velocity index

Evolution from baseline to D28 and M3 will be described only for the patients of the SAF population without liver transplantation using paired Hodges & Lehmann estimator with its 95% confidence interval for the following common clinical laboratory parameters:

- WBC, Neutrophil count, Bilirubin, creatinine, albumin, and CRP

All previous analyses (tables and graphics for general overview and/or on the short term around infusion), except comparison between visits, will be also performed by automated-calculated cell concentration dose cohorts on the SAF population for the following parameters:

- Common clinical laboratory: WBC, Neutrophil count, Bilirubin, creatinine, albumin, and CRP
- Thrombin generation test (central measurements): ETP, Peak and Velocity index

5.2.3 Cytokines analysis

Inflammatory and anti-inflammatory cytokines: at baseline and days 8, 14 and 28 (blood testing in central lab)

All analyses will be provided by planned treatment dose and overall in the SAF and PP populations, unless stated otherwise. The occurrence of liver transplantations will be specified for all tables (footnote) and graphics (symbol or footnote).

Description of each cytokine by class (below LLOD / In range) whatever the visit and only on the SAF population will be provided.

Values at screening and at D1 prior infusion and the change from screening to D1 prior infusion will be summarized:

- Values at screening and at D1 prior infusion and the change from screening to D1 prior infusion for cytokines with at least 80% of values “In range”.
- Shift table of values (below LLOD / In range) at screening and at D1 prior infusion for cytokines with a proportion of values “In range” between 20% (greater or equal) and 80% (lesser).

Values at baseline and each post-baseline time point and corresponding changes from baseline to each post-baseline time point will be summarized:

- Quantitative description will be performed only for cytokines with at least 80% of values “in range”.

- For other cytokines, a description by class (below LLOD / In range) at each visit will be provided if there are at least 20% of values “in range”.

This analyse will be also provided according to the automated-calculated cell concentration dose cohort in the SAF population.

5.2.4 Survival analysis

The percentage of mortality, overall survival and transplant free survival will be analysed at D28, M3 and M12 on the SAF population for all patients, for ACLF patient at baseline and for AD patient at baseline.
Moreover a listing of individual data with indicators for death, overall survival and transplant free survival at time points of interest and ACLF grade at screening and baseline will be provided.

5.2.5 Comparison between naïve and non-naïve to glucocorticosteroid groups

Description of values at baseline, D28 and M3 and change from baseline to D28 and M3 for naïve and non-naïves to glucocorticosteroids groups will be provided on the SAF population for the following parameters:

- New score MELD
- Common clinical laboratory: WBC, neutrophils, lymphocytes, Bilirubin, CRP
- Cytokines: CRP

Difference between naïve and non-naïve to glucocorticosteroids groups will be estimated at baseline, D28 and M3 using a Hodges-Lehmann estimator for independent sample with its 95% confidence interval for the previous parameter.
At D28 and M3, the percentage of mortality, the percentage of overall survival and the percentage of liver transplantation free survival will be also provided for naïve and non-naïve to glucocorticosteroids groups.

5.3 Safety data

5.3.1 Adverse events

All analyses will be provided by planned treatment dose and overall in the SAF population.
The occurrence of liver transplantations will be specified for all tables (footnote).

The number and the percentage of patients having at least the following event and the number of events will be presented at day 28:

- one AE.
- severe AE.
- one SAE.
- one AE/severe AE or SAE related to IMP.
- one AE/severe AE or SAE related to study procedure.

- one AE/severe AE or SAE having led to discontinuation of treatment.
- one SAE having led to death.
- one AE/severe AE or SAE related to IMP having led to discontinuation of treatment.
- one AE/severe AE or SAE related to study procedure having led to discontinuation of treatment.
- one SAE related to IMP having led to death.
- one SAE related to study procedure having led to death.

The number and the percentage of patients having at least the following event (SAE, AESI) will be presented between day 28 and month 3, and between month 3 and month 12.

- one SAE, only for analysis between day 28 and month 3
- one SAE having led to death
- Liver transplantation
- Outcome of transplantation
- Malignancies
- Hospitalisation for ACLF
- One AE possibly related to IMP

The number and percentage of patients presenting at least one AE will be summarized in a frequency table by body system organ class (SOC) and preferred terms (PT) as per MedDRA dictionary. The number of events will be also summarized in the same table. The table will be displayed by descending order based on the number of patients. Each patient will be counted only once within each classification (SOC / PT).

This analysis will be performed

- by SOC, PT at Day28 for all AE, between day 28 and month 3 only for AESI, between month 3 and month 12 only for AESI.
- by SOC, PT, and severity at Day28 for all AE, between day 28 and month 3only for AESI, between month 3 and month 12 only for AESI

All AEs will be listed separately including at least the following items:

- Dose cohort (1a/1b/2a/2b)
- Patient number
- Age / Sex
- Infusion dates
- The preferred term (PT) and the system organ class (SOC)
- The verbatim
- Date of onset and date of end (study day) or ongoing
- Duration of the AE in days
- Intensity

- Outcome
- Relationship to studied pathology
- Relationship to IMP
- Relationship to specific study procedure
- Actions taken regarding the IMP
- Action taken regarding the event
- Evaluation of Seriousness (SAE onset date, seriousness)

Adverse events related to IMP, serious adverse events, adverse events leading to discontinuation, adverse events leading to death, adverse events of special interest will be listed separately.

5.3.2 Laboratory data

All analyses will be provided in the safety population.
The occurrence of liver transplantations will be specified for all tables (footnote).

The values of the parameters and the corresponding changes from baseline to post-baseline values will be summarized by planned treatment dose and overall at baseline and each post-baseline time point.

The status of the results (normal/abnormal not CS / abnormal CS) will be summarized by dose cohort and overall at baseline and each post-baseline time point and for each parameter. Shift tables presenting the status at baseline versus the worst status during study will be presented by planned treatment dose and overall.

The following parameters will be described:

- Common clinical laboratory tests[1] at baseline, days 4, 8, 12, 14, 21, 28, and months 2, 3, 6 and 12.
    - White Blood Cell count, hemoglobin, hematocrit, neutrophils, lymphocytes, monocytes, eosinophils, basophils.
    - platelets (additional measures on infusion day: post infusion at 4h, 24h, 48h, and 72h at day 1, and day 8 in cohort 2b and 2d),
    - GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT (in sec)
    - Serum albumin, sodium, potassium,
    - Blood gas: PaO2, SpO2 and FiO2

[1] For first patients some parameters were not collected in the study

- Coagulation factors[1]: at baseline, and day 8 and additional measures on infusion day (post infusion at 4h, 8h, 12h, 24h, 48h and 72h at day 1, and day 8 in cohort 2b and 2d).
    - INR
    - aPTT (in sec)
    - fibrinogen
    - D-Dimers

- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X): at baseline and day 8 and additional measures on infusion day (24h post infusion at day 1, and day 8 in cohort 2b).

- Thomboelastogram (central measurements only): at baseline and days 4, 8 12, 14, 21 and 28, and additional measures on infusion day (4h post infusion at day 1, and day 8 in cohort 2b and 2d) (blood testing in central lab):
    - Clotting time
    - Clot Formation Time
    - Amplitude 10 min
    - Maximum Clot Firmness
    - Maximum of Lysis

- Thrombin generation test (central measurements): at baseline and days 4, 8 12, 14, 21 and 28, and additional measures on infusion day (4h post infusion at day 1, and day 8 in cohort 2b and 2d) (blood testing in central lab):
    - Lag time
    - ETP
    - Peak
    - Time to peak
    - Velocity index

- Anti-HLA antibodies (class I and class II by luminex method): at baseline, day 28, and month 3, 6 and 12 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at baseline, days 4, 8, 12, 14 and 28 and month 12

- Chest X Ray and Cardiac US Doppler: at baseline and month 12:
  For Cardiac US Doppler only: If abnormal, describe (left-right shunt/Right-left Shunt/No shunt), Pulmonary arterial pressure.

At Minima, for each patient, parameters results, status, and specification of abnormalities will be listed.
"Patient fasting ? (yes/no) and Samples according to study manual (yes/no) ?" will be described for central laboratory measurement in listing only.
The values of laboratory parameters will be summarized by standard unit as defined in SDTM database.

5.3.3 Physical examination

All analyses will be provided by planned treatment dose and overall in the safety population.
The occurrence of liver transplantations will be specified for all tables (footnote).

The status of the results (normal /abnormal) of Physical examination (cardiac, pulmonary, abdomen, upper extremities, lower extremities, neurologic, skin, other) will be summarized by treatment dose and overall and baseline and at each post-baseline time point. Additionally, shift tables presenting the status at baseline versus the worst status during study will be presented by treatment dose and overall.

Physical examination will be described at baseline, days 4, 8, 12, 14, 21 and 28 and months 2, 3, 6 and 12.

At minima, for each patient, parameters results, status, and specification of abnormalities will be listed.

5.3.4 Vital signs

The values of the parameters and the corresponding changes from baseline to post-baseline values will be summarized by planned treatment dose and overall at baseline and each post-baseline time point in the safety population.
The occurrence of liver transplantations will be specified for all tables (footnote).

Vital signs will be described at baseline, days 1, 4, 8, 12, 14, 21 and 28 and months 2, 3, 6 and 12, and additional measurements on infusion day (during and after infusion at day 1, and day 8 in cohort 2b).

At minima, for each patient, parameters results, status, and specification of abnormalities will be listed.

The following parameters will be described: Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation.

5.3.5 IMP administration

The following information will be described by treatment group and overall in the safety population:

- Proportion of patient who completed all planned infusions

- Number of patients who performed each infusion for patients with an administration scheme with repeated infusions
- Duration of IMP exposure: expressed in days and calculated as

  (Date of last IMP infusion) - (date of first IMP infusion) +1
- Total dosage of HepaStem
- For each infusion:
  - Time from Hydrocortisone administration (minutes)
  - Total theoretical dose of HepaStem
  - Total effective dose (actually infused) of HepaStem
  - Effective (actual) dose of HepaStem infused per kg.
  - Intravenous Access (Peripheral/Central)
  - Duration of infusion (min)
  - Minimum Flow rate during infusion (mL/min)
  - Maximum Flow rate during infusion (mL/min)
  - Interruption of the flow rate of HepaStem (Yes/No)
  - Actual volume of HepaStem infused (mL)
  - Infused Volume ratio

The occurrence of liver transplantations will be specified for all tables (footnote).

5.4 Analysis of sub-groups/complementary analysis

All analysis will be performed by planned treatment dose and overall.

6 Tables, Listings and Graphs (TLG)

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STATISTICAL ANALYSIS PLAN

HEP101 – PROMETHERA BIOSCIENCES

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Final version 4.0
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1 List of abbreviations and definition of terms

Abbreviations	Definitions
ACLF	Acute on Chronic Liver Failure
AD	Acute Decompensation
AE	Adverse Event
AESI	Adverse Event of Specific Interest
aPTT	Activated Partial Thromboplastin Time
BUN	Blood Urea Nitrogen
CLIF-C	CLIF Consortium
CRP	C-Reactive Protein
CS	Clinically Significant
ECG	Electrocardiogram
ETP	Endogenous Thrombin Potential
FAS	Full analysis set
FiO2	Fraction of Inspired Oxygen
GOT	Glutamic Oxaloacetic Transaminase
GPP	Good Pharmacoepidemiology Practices
GPT	Glutamate Pyruvate Transaminase
γGT	γ Glutamyl Transferase
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HE	Hepatic Encephalopathy
HEV	Hepatitis E virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
KBP	Keyrus Biopharma
LLOD	Lower Limit Of Detection
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
MSC	Mesenchymal Stem Cell
Na	Sodium
NCS	Not Clinically Significant
OF	Organ Failure
PaO2	Partial Pressure of Oxygen in Arterial Blood
PP	Per protocol population
PT	Preferred Terms (MedDRA dictionary)
SAE	Serious Adverse Event
SAF	Safety population
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDTM	Study Data Tabulation Model

SMC	Safety monitoring committee
SpO2	Pulse Oximeter Oxygen Saturation
SOC	System Organ Class (MedDRA dictionary)
TEG	Thromboelastogram
TLG	Tables, Graphs and Listings
WHO	World Health Organization

2 Introduction

This Statistical Analysis Plan (SAP) determines the frame of statistical analysis of this study in agreement with the protocol version 1.0 for Belgium dated on March 23nd 2016, the protocol version 2.0 for Belgium dated on December 13th 2016, the protocol version 3.2 dated May 11th 2017 for Belgium, the protocol version 4.0 dated on February 15th 2018 in Bulgaria and Spain, the protocol version 5.0 dated on June 26th 2018 in Belgium, the protocol version 5.1 dated on June 26th 2018 in France and the protocol version 6.0 dated on December 14th 2018 in Belgium and the protocol version 6.1 dated on February 05th, 2019 in France.

The SAP is reviewed, approved, and signed by the Biostatistician and Sponsor prior to database lock, at the latest.

This document will be the main reference document as far as statistical analyses are concerned.

The purpose of this document is to describe:

- The study features as per protocol in terms of objectives, study design and study conduct
- The endpoints, the study cohorts, the study variables and the derived data
- The planned statistical analysis and methodologies.

3 Study description

3.1 Study objective

3.1.1 Primary objectives

To assess the safety of different dose regimens of HepaStem in cirrhotic Patients with ACLF or with acute decompensation at risk of developing ACLF up to Day 28 of the active study period.

3.1.2 Secondary objectives

- Preliminary efficacy:

To evaluate clinical and biological efficacy parameters following HepaStem infusion up to Day 28 and up to Month 3 and Year 1 post first HepaStem infusion.

- Long term safety:

To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

3.2 Study design

This is an interventional, multicenter, open-label, non-controlled prospective study of different dose regimens of HepaStem given in subsequent cohorts.

Initially, according to protocol version 1.0 and 2.0, patients with CLIF-ACLF grade 1 or grade 2 were to be included. Patients in the first cohort (6 patients) were planned to receive 4 HepaStem infusions over 2 weeks at a dose of 250 x 10^6 cells per infusion (approximatively 3.5 x 10^6 cells/kg). Patients in the second cohort (6 patients) were planned to receive a double dose with the same schedule. However, in the first cohort (referred as cohort 1a), 2 bleeding events were assessed as SAEs causally related to HepaStem by the Investigator. The bleeding was successfully managed, and both patients recovered.
Following 2 SAEs of severe bleeding observed in 2 ACLF patients with pre-existing coagulation disturbances (1 of the same type in each patient) which led to a safety signal, changes were included in protocol version 2 in order to increase the safety of the study (1) the inclusion criteria were modified to allow inclusion of less severely-ill patients for the next 3 patients; 2) the dose was reduced (proposed 5x reduction of dose/infusion) for the next 3 patients, with an increase in dose for the next cohorts, (3) implementation of additional safety measures with regular blood test for coagulation status before, during and after infusion for up to 24 hrs or longer if medically indicated, (4) implementation of stepwise patient inclusion, the data of the next 3 patients to be reviewed by the SMC.
Three less severely ill patients (ACLF grade 0) received a single infusion of 0.25 x 10^6 cells/kg (cohort 1b). Following consultation of the SMC members, enrolment into the next dose cohort could begin: 3 patients received single infusion of 0.5 x 10^6 cells/kg (cohort 2a), and 3 patients received 2 infusion of 0.5 x 10^6 cells/kg (cohort 2b).
Following consultation of the SMC members, inclusion of more severely ill patients was allowed, as well as the dose increase for additional cohorts 2c and 2d (single or double infusion of 1.0 x 10^6 cells/kg per infusion). Four more patients received 2 infusion of 0.5 x 10^6 cells/kg (cohort 2b), 3 patients received a single infusion of 1.0 x 10^6 cells/kg (cohort 2c) and 5 patients received two infusions of 1.0 x 10^6 cells/kg per infusion (cohort 2d).

The recruitment is completed (24 patients in total), and the Safety follow-up is ongoing, with an anticipated LSLV in July 2020.

The study is divided in the following periods: screening period, active study period dived in treatment period and evaluation period, and long-term safety follow-up.

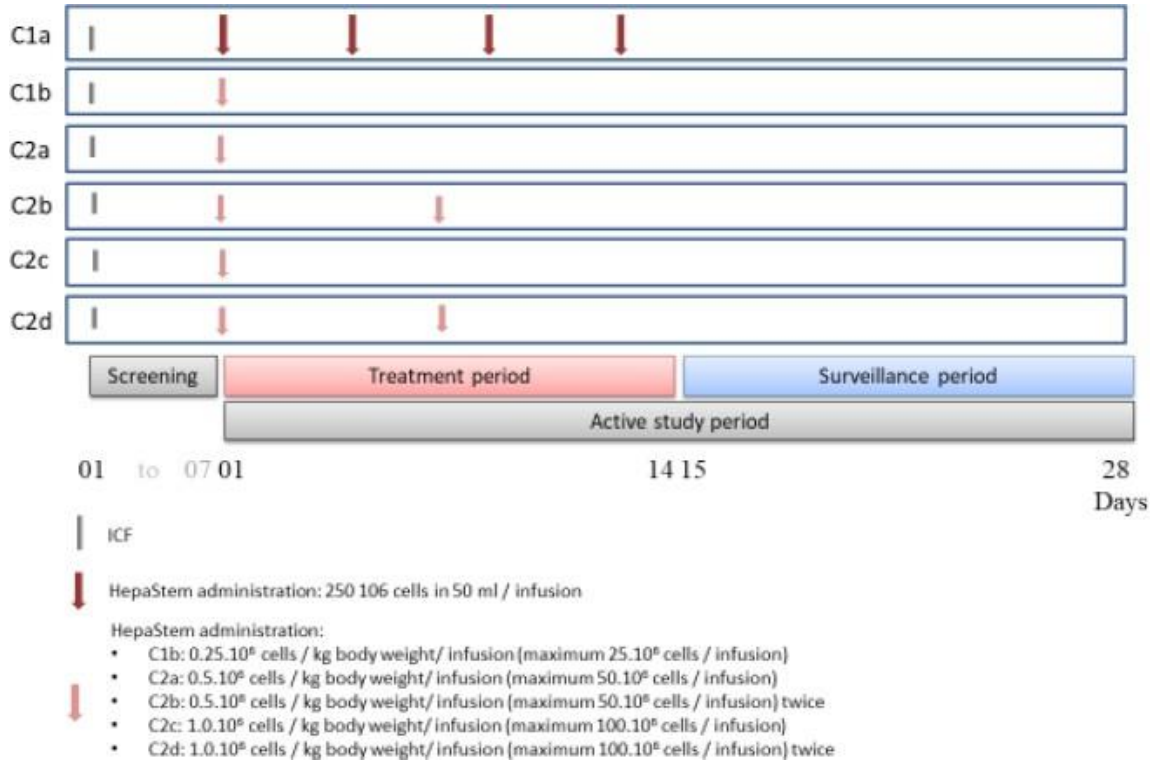
Screening period: Once informed consent is signed, may last maximum 7 days, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria.

Active study period: The active study period lasts 28 days (± 2 days). The duration of the screening period plus the active period lasts up to 35 days (±2 days).

Enrolled patients not meeting the inclusion/exclusion criteria are considered as screening failure and will be replaced. Any patients receiving at least one HepaStem infusion is considered as an included and evaluable patient.

Various dose regimens of HepaStem have been given, which differ in the amount of cells per infusion as shown in Figure 1.

Figure1 -Study scheme of active study period



Long-term safety follow-up: After completion of the active study period, patients are followed-up up to 1 year post first HepaStem infusion in the safety follow-up period.

After completion of this study, patients will be invited to participate in a long-term safety follow-up study for 5 years (PROLONGSTEM).

3.3 Statement on the changes in measuring cell concentration:

During the course of the study HEP101, Promethera recognized a need to standardize the methods of measuring the doses of cells for clinical use, which was based on different methods of counting the cells manually during the preparation of the batches. Indeed, during the past years, and during the study HEP101, different non-validated manual methods were used to determine the concentration of cells in Total Cell Count (TCC)/mL. This led to inconsistencies in the dosing for clinical use, including for the use in HEP101. Promethera decided to apply another method of cell counting, the NC-200 automate counter, a fully validated method for determining the TCC/mL.
In addition, Promethera decided to change the test items for TCC/mL determination. For the study HEP101, the TCC/mL was measured during the preparation of the batched, on the drug product formulated and filled before freezing. The new method of counting is now applied on the drug product after thawing and reconstitution, as it represents the actual product quality to be administered to the patients. The NC-200 automated method after thawing and reconstitution allows for a more accurate cell concentration than with the former manual methods before freezing.
This new NC-200 method of quality control has been applied on the historical batches used for HEP101, and the cell concentration has been re-assessed after thawing and reconstitution.
Differences have been observed between the intended cell concentration assessed before freezing (manually calculated TCC/mL) and the determined cell concentration after thawing and reconstitution (automated calculated TCC/mL). The differences are explained by the differences between the old manual cell count methods used to adjust the cell concentration for formulation before freezing as compared to the new defined automated cell count method used after thawing and reconstitution.

Based on information provided by the sponsor, the Automated calculated cell concentration for each patient has been listed in a table in the minutes of data review meeting n°3 Dated 28 october 2019. This table have been reported in an excel file, that will be integrated in the ADaM database.

Automated-calculated cell concentration dose cohorts have been created based on the range of automated calculated cell concentration and the number of infusion performed. Consequently, patients could be regrouped according to the following class:

- Patients of cohort 1a
- Patients with Automated-calculated TCC/mL dose between 0.6 and 0.8 million cells / kg bodyweight and 1 infusion
- Patients with Automated-calculated TCC/mL dose of 0.6 million cells / kg bodyweight and 2 infusions
- Patients with Automated-calculated TCC/mL dose of 1.2 million cells / kg bodyweight and 1 infusion
- Patients with Automated-calculated TCC/mL dose of 1.2 million cells / kg bodyweight and 2 infusions

3.4 Study plan

During the screening and treatment period, patients are hospitalised in intermediate or ICUs or standard units, depending of the severity of the patient disease.
Once informed consent is signed, the screening period may last from 1 to 4 days for protocol version 1, 2, 3.1 and 3.2 and up to 7 days for protocol versions 4.0, 5.0, 5.1, 6.0 & 6.1, the time to complete the screening process and to deliver HepaStem. The screening period aims at assessing the inclusion and exclusion criteria. During the screening period, comprehensive medical history is recorded, such as background condition leading to cirrhosis, possible previous episode(s) of Acute Decompensation and/or ACLF, significant background condition, relevant medications, and factor(s) triggering Acute Decompensation and/or ACLF. The examinations listed below must be performed at least once. Before initiating HepaStem treatment, for each patient, the main eligibility criteria are reviewed and discussed with the medical monitor of Promethera to ensure patient eligibility.

Patients are treated in a stepwise approach.

During the treatment period, a study visit is performed on Days 1, 4, 8, 12 and 14 (± 2 days for each visit form the Day 4 visit) including the evaluation listed in table 2.

On HepaStem infusion days before each infusion, a physical exam, evaluation of vital signs, blood tests including a coagulation evaluation with INR, fibrinogen, platelet, aPTT, coagulation factors (from protocol version 3.0), TEG (if already performed as a part of the clinical routine and up to investigator's judgment), a liver echography and Doppler, and evaluation of disease scorings is performed, as listed below. These assessments are performed on each planned infusion day even if HepaStem infusions are prematurely stopped.

The careful evaluation of these parameters allows or not the infusion or treatment discontinuation. Vital signs evaluation is also performed during and after infusion and, from the protocol version 3.0, coagulation parameters and TEG are closely monitored after infusion as listed in study flowchart.

On the other days during the hospital stay, patients are followed-up according to usual practice.

After the treatment period, study visits are done on days 21 and 28 (± 2 days for each visit), as outpatients for those discharged, or as inpatients for those not discharged.

After the active study period, patients enter the follow-up period up to 1 year, including visits at Month 2 (± 2 weeks), Month 3 (± 2 weeks), Month 6 (± 2 weeks), and Year 1 (± 1 month).

Up to the 28 days visit, all SAEs are collected. After the 28 days visit, up to Month 12 visit, safety is based on collection of AE of Special Interest (AESI) including SAE with fatal outcome, liver transplantation, malignancies, new Acute Decompensation and/or ACLF episode, AEs assessed by the investigator as possible related to HepaStem.
At the Month 12 study visit, patients will be invited to be included in the long-term safety follow up study (5- years).

Table 2 Study Flowchart

Period	Screening Period	Active period							Long term follow-up			
	Baseline	Treatment Period					Surveillance Period					
Time	Over 1-7 days prior D1	Infusion D1	D4 ± 2 days	D8 ± 2 days	D12 ± 2 days	D14 ± 2 days	D21 ± 2 days	D28 ± 2 days	M2 ± 2 weeks	M3 ± 2 weeks	M6 ± 2 weeks	M12 ± 1 month
Informed Consent	X											
Eligibility criteria	X											
Demography & Medical History	X											
Physical exam	X	Xa	X	Xa	X	X	X	X	X	X	X	X
Vital Sign	X	Xb	X	Xb	X	X	X	X	X	X	X	X
Scoring : West-Haven HE, CLIF-OF, CLIF-C ACLF, ACLF grade, CLIF-C AD, MELD, Child Pugh score	X	X	X	X	X	X	X	X	X	X	X	X
Biological analysis												
WBC, Platelets, RBC, CRP, Na+, K+, Alb, Creat, Urea/Bun	X	X%	X	X%	X	X	X	X	X	X	X	X
GOT, GPT, Bilirubin, Alk Ph, γGT	X	X%	X	X%	X	X	X	X	X	X	X	X
Lipase & Coagulation 2: C-protein, S-protein, Anti-Thrombin III	X											
Coagulation 1 : INR, aPTT	X	X+	X	X+	X	X	X	X	X	X	X	X
Virology status (HbS Ag, HCV, HEV, HIV), Aspergillosis test	X											
Coagulation 3 : Fibrinogen	X	X+		X+								
Coagulation 3 : D-Dimers, optional local TEG		X+		X+								
Coagulation factors : Intrinsic pathway (VIII, IX, XI, XII) and extrinsic pathway (II, V, VII, X)	X	X&		X&								
Urine analysis : Sediment, Creat, Glc, Protein, Albm	X											
Plasma samples (Central Lab)												
Cytokines	X	Xa		Xa		X		X				
TEG, TG	X	X*	X	X*	X	X	X	X				
Anti-HLA antibodies (cl 1, cl 2)	X							X		X	X	X
Imaging / Radiology & ECG												
Abdominal & portal system US Doppler	X	Xa	X	Xa	X	X	X	X				X
Chest X-Ray	©											X
ECG	©											
Cardiac US Doppler	©	≠										X
Blood culture or other fluid culture	c											
Investigational Product : HepaStem Infusions[a]												
Cohort 1b : Infusion of 0.25.10⁶cells /kg body weight with a maximum of 25.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X										
Cohort 2a & b : Infusion of 0.5.10⁶ cells /kg body weight with a maximum of 50.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2b only								
Cohort 2c & d : Infusion of 1.0.10⁶ cells /kg body weight with a maximum of 100.10⁶ cells + Hydrocortison (100mg) 15-30min prior infusion		X		Cohort 2d only								
Concomitant medication & therapy	Continuously								Relevant			
Safety (Aderse Events)	All AEs								AESI			

a: On infusion day: before infusion
b: On infusion day: before, during and after infusion
C: Only if performed during the same admission
%: On infusion day: all parameters are measured prior infusion /platelets measurement to be performed prior and post infusion at 4h, 24h, 48h and 72h.
+: On infusion day: prior and 4h, 8h, 12h, 24h, 48h and 72h post infusion
&: On infusion day: prior and 24h after infusion
©: if not already performed during same admission. If already performed, results collected
*: On infusion day: prior and 4h after infusion
≠: cardiac US to be performed after infusion
AESI: only Adverse Event of Special Interest to be reported

3.5 Changes in the conduct of the study

Cf; amendment

4 Statistical methods

4.1 General statistical considerations

All statistical analyses will be performed with the SAS software version 9.4.

Being an exploring efficacy and safety study, no formal statistical hypotheses will be assessed.

Standard descriptive statistics will be used for quantitative and categorical variables.

Quantitative variables will be presented using the number of observed values, number of missing observations, mean, standard deviation, and median, minimum and maximum.
When required, Confidence intervals will be computed based on the Wald method.

Evolution between baseline and post-baseline visits will be estimated using the non-parametric approach of Hodges & Lehmann for paired sample. Estimate and its two-sided 95% confidence interval of the difference between visits will be provided.

Comparison between groups will be estimated using the non-parametric approach of Hodges & Lehmann for independent samples. Estimate and its two-sided 95% confidence interval of the difference between groups will be provided.

Categorical variables will be presented using counts and percentages of patients. The number of missing observations will also be presented.
When required, Confidence intervals will be computed based on the Clopper-Pearson method.
Percentages, based on the non-missing data, will be presented with one decimal.

All listings will be presented in appendix 16.2 and sorted by site number, subject number, except in specific cases.

All statistical analyses, except comparison, will be described by planned dose cohort and overall.

AEs will be coded to a preferred term and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA).

Prior and concomitant medications and treatments will be coded using the World Health Organization (WHO) Drug Dictionary.

Two interim analyses will be performed on patients with consent signed between the December 5th 2016 and the September 17th 2018 included. First interim analysis will be performed after completion of the 28 days active study period (or have died or have been lost to follow-up) and the second after completion of 3 months follow-up period (or have died or have been lost to follow-up).
Two other interim analyses will be performed on all patients enrolled in the study. The third interim analysis will be performed after completion of the 28 days active study period (or have died or have been lost to follow-up) and the fourth after completion of 3 months follow-up period (or have died or have been lost to follow-up.

For interim analyses, cut-off date corresponds at the date of last visit for each period of interim analysis. If the last visit of the considering period is not performed, then the theoretical date will be used. The cut-off date will different according to the patient.
Management of data selection according to the cut-off date will be made as follow:

Data obtained after the cut-off will not be displayed in any listings or used for summary statistics, e.g. laboratory values of samples taken after data cut-off, AE with onset date after data cut-off, etc. will not be included in any analysis or listing.
For the following data, the following special rules apply for the derivation of the variables:

- Date of discontinuation after date of cut-off: Date and reason of discontinuation will be set to missing.
- Death later than date of cut-off: Date of death will be set to missing
- Last date known to be alive later than date of cut-off will be replaced by date of cut-off
- For adverse event occurred before or the day of cut-off, no information will be removed or modified
- For concomitant medication started before or the day of cut-off, no information will be removed or modified

Final analysis will be performed after completion of 1 year follow-up period (or have died or have been lost to follow-up) for all enrolled patients in the study.

For SMC meeting, the members will be provided with reports which will include data on recruitment, safety assessment data, and any other data as required by the SMC Members.

4.2 Sample size calculation

No sample size calculation was performed.
The total sample size depended on the recommendations given by SMC in order to protect patient safety based on a risk assessment.
In total, 24 evaluable patients were included.

4.3 Population Analysis

4.3.1 Full analysis set (FAS)

The FAS population will include all patients screened in the study. This population will be analysed only for the disposition table. A patient will be considered as screen-failure if the eligibility has not been approved by the medical monitor or the patient did not meet the inclusion/exclusion criteria.

4.3.2 Safety population (SAF)

The safety population will include all patients entered into the study who received at least one dose of IMP. This population will be the primary dataset for all safety and efficacy analyses.

4.3.3 Per-Protocol (PP) population

The per-protocol (PP) population will include patients from the safety population without any major protocol deviation. This population will be another dataset for all efficacy analyses.

4.4 Protocol deviations

All potential deviations in relation to the study protocol and inclusion criteria of patients will initially be defined in details and searched within the database before the pre-analysis data review meeting. Results of these deviations will be reviewed on a patient-by-patient basis at the Data Review Meeting.

Protocol version 1.0 and 2.0 (Cohort 1a):

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old
2. Informed Consent.

N.B: In case of hepatic encephalopathy, Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by Liver histology or Clinical and imaging examination (may include fibroscan).
4. ACLF Grade 1 or ACLF Grade 2 with the following restrictions:

ACLF grade 1 eligible subset:

- liver failure plus cerebral and/or kidney dysfunction

- renal failure plus cerebral dysfunction
- cerebral failure plus kidney dysfunction
- coagulation failure plus cerebral and/or kidney dysfunction

Or

ACLF grade 2 eligible subset:

- Any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure.

Organ dysfunctions or failures are defined according to CLIF-C OF score as below

Diagnostic criteria of kidney and cerebral dysfunction
kidney: moderate impairment in renal function as defined by a serum creatinine ranging from 1.5 to 1.9 mg/dL.
cerebral: moderate impairment of brain function as defined by grade I-II HE based on West Haven criteria.
Diagnostic criteria of organ failures
liver: serum bilirubin ≥ 12 mg/dL;
kidney: serum creatinine ≥ 2 mg/dL;
cerebral: grade III-IV HE based on West Haven criteria;
coagulation: international normalized ratio [INR] ≥ 2.5

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.
2. Known prothrombotic disease or medical history of thrombotic events.
3. Gastrointestinal haemorrhage requiring blood transfusion unless controlled for more than 48h.
4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.
5. Clinical evidence of *Aspergilus* infection.
6. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressine to control increased levels of creatinine is not an exclusion criterion.
7. Respiratory disordered with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.
8. Treatment with corticosteroids for acute liver disease within 2 weeks before HepaStem infusion.
9. MELD score > 35.
10. Previous organ transplantation and/or ongoing immunosuppressive treatments.
11. Postoperative-decompensation following hepatectomy.

12. Renal failure due to chronic kidney disease.
13. Clinically significant left-right cardiac shunt.
14. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.
15. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.
16. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.
17. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.
18. Participation to any other interventional study within the last 4 weeks.
19. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

Protocol version 3.2 and 4.0 (Cohort 1b, 2a, 2b):

- Inclusion criteria:

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfill all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.

2. Informed Consent.

N.B: In case of hepatic encephalopathy, if the patient is not able to fully understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

- liver histology or

- clinical and imaging examination (may include fibroscan).

4. Patient with Acute Decompensation of cirrhosis

5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)

6. The INR measurement has to be: $1.2 \leq INR < 2$

- Exclusion criteria

Any of the following criteria will exclude a patient from the study:

1. Absence of portal vein flow as assessed by Doppler ultrasound or other exam.

2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion. Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.

3. Gastrointestinal hemorrhage requiring blood transfusion unless controlled for more than 4 weeks prior to the first infusion.

4. Variceal banding or sclerosis within 4 weeks before the infusion

5. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.

6. Clinical evidence of *Aspergilus* infection.

7. Circulatory failure defined as treated with vasoconstrictors to maintain arterial pressure or inotropes to improve cardiac output. N.B: Use of terlipressin to control increased levels of creatinine is not an exclusion criterion.

8. Respiratory disorders with pulse oximetry < 93% and related clinical signs, requiring or not mechanical ventilation.

9. Coagulation disorders defined as:

- INR ≥ 2

- Fibrinogen < 100 mg/dL

- Platelets < 50.000/mm^3

10. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator.

11. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.

12. MELD score > 30.

13. Previous organ transplantation and/or ongoing immunosuppressive treatments.

14. Postoperative-decompensation following hepatectomy.

15. Renal failure due to chronic kidney disease.

16. Clinically significant left-right cardiac shunt.

17. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.

18. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years.

19. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.

20. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.

21. Participation to any other interventional study within the last 4 weeks.

22. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

All decision concerning a potential withdrawal of a subject from a population of analysis due to protocol deviations will be discussed with the sponsor before data base lock. If necessary, the last version of this statistical analysis plan will be updated accordingly.

Protocol version 5.0, 5.1, 6.0 and 6.1 (Cohort 2b, 2c, 2d):

- Inclusion criteria:

Before initiating HepaStem treatment, for each patient, the main eligibility criteria will be reviewed and discussed with the medical monitor of PROMETHERA to ensure patient eligibility.

A patient must fulfil all of the following criteria to be eligible to participate in the study:

1. Adult aged between 18 and 70 year old.

2. Signed Informed Consent.

N.B: In case of hepatic encephalopathy, if the patient is not able to understand the study based on the investigator's judgment, the Informed Consent must be signed by patient's legal representative according to local regulation, and by the patient, if possible, after encephalopathy improvement.

3. Cirrhosis as diagnosed by

- liver histology or

- clinical and imaging examination (may include fibroscan).

4. Patient with Acute Decompensation of cirrhosis

5. Serum total bilirubin≥ 6 mg/dL (≥100 umol/L)

- Exclusion criteria

Any of the following criteria will exclude a patient from the study:

1. Thrombosis of the portal vein.

2. Recurrent or ongoing thrombotic events or patients considered with high risk of thrombosis at the time of infusion.

Any thrombosis history should be discussed with the medical monitor before exclusion / inclusion in the study, in a case by case discussion.

3. Ongoing uncontrolled bleeding.

4. Septic shock or non-controlled bacterial infection defined as persistent clinical signs of infection despite adequate antibiotic therapy for more than 48h.

5. Clinical evidence of Aspergillus infection.

6. Circulatory failure defined by inability to maintain a mean Blood pressure ≥ 70 despite use of vasopressors

7. Mechanical ventilation due to respiratory failure.

8. Coagulation disorders defined as:

- Fibrinogen < 80 mg/dL

- Platelets < 40.000/mm3

9. Major invasive procedure within 4 weeks before the infusion (within 1week for minor invasive procedure such as e.g. transjugular liver biopsy). The proper healing of the puncture site should be verified by the investigator.

10. Treatment with corticosteroids for acute liver disease less than 1 day before the start of the screening period.

11. MELD score > 35.

12. Previous organ transplantation and/or ongoing immunosuppressive treatments.

13. Postoperative-decompensation following hepatectomy.

14. Renal failure due to chronic kidney disease.

15. Clinically significant left-right cardiac shunt.

16. Known or suspected hypersensitivity or allergy to any of the components of the HepaStem Diluent (human albumin, heparin sodium and sodium bicarbonate) or a history of multiple and/or severe allergies to drugs or foods or a history of severe anaphylactic reactions.

17. Malignancies, other than curatively treated skin cancer, unless a complete remission over 5 years. In case of suspicion of HCC, all exam should be done to confirm or not the diagnosis prior enrolment.

18. Refusal of abstinence from alcohol for at least 5 weeks from the study enrolment.

19. Pregnancy (negative β-HCG test required) or women with childbearing potential who decline to use reliable contraceptive methods during the study.

20. Participation to any other interventional study within the last 4 weeks.

21. Any significant medical or social condition or disability that, in the Investigator's opinion, may warrant a specific treatment, or may interfere with the patient's optimal participation or compliance with the study procedures.

All decision concerning a potential withdrawal of a subject from a population of analysis due to protocol deviations will be discussed with the sponsor before data base lock. If necessary, the last version of this statistical analysis plan will be updated accordingly.

4.5 Handling of missing values

For the start dates of Adverse Event, missing dates won't be replaced, however following rules will be applied for the classification of Adverse Events as emergent/non-emergent (ie AE occurs during or outside the study period):

- if start day is missing, and start month/year is inferior to first treatment infusion, then Adverse Event will be considered as non-emergent
- if start day is missing, and start month/year is superior or equals to first treatment infusion, then Adverse Event will be considered as emergent
- if start day and start month are missing, and start year is inferior to first treatment infusion, then Adverse Event will be considered as non-emergent
- if start day and start month are missing, and start year is superior or equals to first treatment infusion, then Adverse Event will be considered as emergent
- if start day, month and year are missing, then Adverse Event will be considered as emergent

For the start dates of medical history:

- if only start day is missing, then the 1rst day of the month will be taken for the imputation
- if start day and start month are missing, then the 1rst January will be taken for the imputation

Other missing data are not replaced and, in generally, not considered in the calculation of the percent.

4.6 Derivate variables

Age (years): (First consent date (by patient or legal representative) – Date of birth)

BMI (kg/m²): weight (kg) / height (m)²

Duration of inclusions (months): (Last patient included date - First patient included date)/30.44

Duration of study (months): (Last patient out date - First patient included date)/30.44

Time since X diagnosis (months): (First administration date – Date of diagnosis)/30.44

Serious Adverse Event (SAE):
The SAEs are the adverse events with the item "Serious Adverse Event" ticked "Yes". If the item is missing, the Adverse Event will be considered as Serious.

AEs related to the IMP:
The AEs related to the IMP are the AEs with the item "Relation to IMP" ticked "Definite Related", "Probable Related", "Possible Related" or missing or unknown.

AEs related to the specific study procedure:
The AEs related to the specific study procedure are the AEs with the item "Relation to specific study procedure" ticked "Definite Related", "Probable Related", "Possible Related" or missing or unknown.

AE leading to discontinuation
The AEs leading to discontinuation are the AEs with the item "Action taken regarding study drug" ticked: "drug interrupted" or "drug withdrawn"

AE leading to death:
The AE leading to death are the AEs with the item Outcome ticked "Fatal"

AE of Special Interest (AESI):
AESI are collected after D28 visit and correspond to AE with one of the following items ticked:

- AEs with fatal outcome
- Liver Transplantation and outcome of the transplantation
- Malignancies
- Hospitalization for ACLF
- AEs assessed by the investigator as possibly related to HepaStem

Liver transplantation:
AE liver transplantation corresponds to AE with "Liver transplant" as PT name.

Thrombin Generation Test: Negative values will be substituted by 0.

Baseline:
Last non missing value before first IMP intake (except for height).

Change (absolute) at each time points: Value at each time points (first value after administration should be considered, recheck will be used only for specific case to justify) – Baseline value (value just before administration recheck included if applicable)

CLIF organ failure score system (CLIF-OF score):

CLIF-Organ Failure score system.

Organ / System	**Sub-score = 1**	**Sub-score = 2**	**Sub-Score = 3**
Liver	Bilirubin < 6mg/dL	6 ≤ Bilirubin ≤ 12mg/dL	Bilirubin >12mg/dL
Kidney	Creatinine <2mg/dL	2 ≤ Creatinine<3·5 mg/dL	Creatinine ≥3·5 mg/dL, renal replacement therapy
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2·0	2·0 ≤ INR < 2·5	INR ≥ 2·5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO2/FiO2 or SpO2/FiO2	 >300 >357	 ≤300 - > 200 >214- ≤357	 ≤200 ≤214

For respiratory score:

- If FiO2 unit corresponds to %, used FiO2 value /100 for score calculation
- If blood gas parameters are not available then pulse oximetric saturation parameter will be used.

Statistical analysis will be based on derivate variables. Sub score can only be calculated based on individual components measured at the same date than the score calculation date. Date associated to each derivate variable, if calculated, corresponds to date of page score in eCRF.

CLIF-C OF score:

Diagnostic criteria of organ failures
Liver: serum bilirubin > 12 mg/dL;
Kidney: serum creatinine ≥ 2 mg/dL;
Cerebral: grade III-IV HE based on West Haven criteria;
coagulation: international normalized ratio [INR] ≥ 2.5
Use of vasopressors for circulatory failure
Respiratory failure with PaO2/FiO2 ≤200 or SpO2/FiO2 ≤214

Statistical analysis will be based on derivate variable. Score can only be calculated based on individual components measured at the same date than the score calculation date. Date associated to derivate variable, if calculated, corresponds to date of page score in eCRF.

ACLF grading system (ACLF grade)

ACLF grade	Organ failure
No ACLF/ ACLF grade 0	- No organ failure - One organ failure (liver, coagulation, circulatory or respiratory failure) with serum creatinine level <1.5 mg/dL and no hepatic encephalopathy. - Single cerebral failure and serum creatinine level <1.5 mg/dL
ACLF grade 1	- Single kidney failure without mild or moderate hepatic encephalopathy - Single organ failure with serum creatinine ranging from ≥1.5 to 1.9 mg/dL) and/or mild-to-moderate hepatic encephalopathy
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3	- Presence ≥ 3 organ failures

Grade will be calculated using CLIF-OF derivate variables.
Statistical analysis will be based on derivate variable. Score can only be calculated based on individual components measured at the same date than the score calculation date
Date associated to derivate variable, if calculated, corresponds to date of page score in eCRF.

West Haven Criteria of Altered mental statins in Hepatic Encephalopathy

Stage	Consciousness	Intellect and Behavior	Neurologic Findings
0	Normal	Normal	Normal examination; impaired psychomotor testing
1	Mild lack of awareness	Shortened attention span; impaired addition or subtraction	Mild asterixis or tremor
2	Lethargic	Disoriented; inappropriate behavior	Muscular rigidity and clonus; Hyperreflexia
3	Somnolent but arousable	Gross disorientation; bizarre behaviour	Muscular rigidity and clonus; Hyperreflexia
4	Coma	Coma	Decerebrate posturing

CLIF-C ACLF SCORE
Not applicable for ACLF grade 0
CLIF-C ACLF = $10 \times [(0.33 \times \text{CLIF OF} + 0.04 \times \text{Age\{years\}} + 0.63 \times \text{Ln(WBC\{}10^9 \text{ cells/L\})} - 2]$
Derivate variables will be used for ACLF grade and CLIF-OF score
Statistical analysis will be based on derivate variable
Score can only be calculated based on individual components measured at the same date than the score calculation date. Date associated to derivate variable, if calculated, corresponds to date of page score in eCRF.

CLIF consortium acute decompensation score (CLIF-C AD):
CLIF-C AD = $10 \times [(0.03 \times \text{Age \{years\}} + 0.66 \times \text{Ln(Creatinine\{mg/dL\}} + 1.71 \times \text{Ln(INR)} + 0.88 \times \text{Ln(WBC\{}10^9 \text{ cells/L\})} - 0.05 \times \text{Sodium \{mmol/L\}} + 8]$

For patients of cohort 1a, CLIF-C AD score was not available in eCRF and will be calculated.
Statistical analysis will be based on derivate variable.
Score can only be calculated based on individual components measured at the same date than the score calculation date. Date associated to derivate variable, if calculated, corresponds to date of page biological analysis in eCRF.

'Initial' or original MELD score:
MELD score is calculated using serum bilirubin in mg/dL, serum creatinine in mg/dL, and International Normalized Ratio (INR) and is given by the formula:

Initial MELD (i) = (0.957 * ln(Serum Creatinine) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10

Management of lower and upper values:

- if serum bilirubin < 1 then serum bilirubin is replaced by 1 for score calculation
- if serum creatinine < 1 then serum creatinine is replaced by 1 for score calculation
- if INR < 1 then INR is replaced by 1 for score calculation
- for patient with hemodialysis, serum creatinine is replaced by 4 for score calculation

Score can only be calculated based on individual components measured at the same date than the score calculation date. Date associated to derivate variable, if calculated, corresponds to date of page biological analysis in eCRF.

'New' MELD Score (2016)
MELD Score (2016) is calculated with same information used in Initial MELD score with Na in mmol/L in addition.
New MELD Score (2016) = Initial MELD (i) + 1.32*(137-Na) – [0.033*Initial MELD (i)*(137-Na)]

Management of lower and upper values:

- if Na <125 then Na, is replaced by 125 for score calculation
- if Na >137 then Na, is replaced by 137 for score calculation
- if MELD(i) < =11 then MELD Score (2016) = MELD(i)

Statistical analysis will be based on derivate variable MELD Score (2016)
Score can only be calculated based on individual components measured at the same date than the score calculation date. Date associated to derivate variable, if calculated, corresponds to date of page biological analysis in eCRF.

Child–Pugh:

SCORE Measure	1 point	**2 points**	**3 points**
Total bilirubin, µmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin (g/dL)	>3.5	2.8–3.5	<2.8
Prothrombin time prolongation (s) Or INR	<4.0 <1.7	4.0–6.0 1.7-2.3	> 6.0 >2.3
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Statistical analysis will be based only on investigator variable. Score can only be calculated based on individual components measured at the same date than the score calculation date. Date associated to derivate variable, if calculated, corresponds to date of page score in eCRF.

Child–Pugh class:
Chronic liver disease is classified into Child–Pugh class A to C, employing the added score from above.

Points	Class	One year survival	Two year survival
5–6	A	100%	85%
7–9	B	81%	57%
10–15	C	45%	35%

Classes will be defined according to derivate variable of Child-Pugh calculated with INR parameter.

FIB-4:
FIB-4 = (age * AST) / (platelet count * SQRT(ALT))
With age in year, ALT and AST in IU/L and platelet count in 10^9/L.
Score can only be calculated based on individual components measured at the same date than the score calculation date. Date associated to derivate variable, if calculated, corresponds to date of page biological analysis in eCRF.

APRI:
APRI = ((AST / AST Upper level of normal) / Platelet count)) * 100
With AST and AST Upper level of normal in IU/L and platelet count in 10^9/L
Score can only be calculated based on individual components measured at the same date than the score calculation date. Date associated to derivate variable, if calculated, corresponds to date of page biological analysis in eCRF.

Cytokines:

The Lower Limit of Detection (LLOD) for each cytokine is indicated in the following table:

Parameter	LLOD (pg/mL)
CRP	0,83
Eotaxin	3,26
Eotaxin-3	1,77
GM-CSF	0,16
IFN-γ	0,37
IL-1α	0,09
IL-1β	0,05
IL-2	0,09
IL-4	0,02
IL-5	0,14
IL-6	0,06
IL-7	0,12
IL-8	0,07

IL-8 (HA)	95,6
IL-10	0,04
IL-12/IL-23p40	0,33
IL-12p70	0,11
IL-15	0,15
IL-16	2,83
IL-17A	0,31
IP-10	0,37
MCP-1	0,09
MCP-4	1,69
MDC	1,22
MIP-1α	3,02
MIP-1β	0,17
TARC	0,22
TNF-α	0,04
TNF-β	0,08
VEGF-A	1,12

The LLOD is applied on diluted sample.
Only final concentration, corresponding to mean of results on diluted sample * dilution factor, will be analysed.
Final concentrations reported as "NaN" correspond, in this study, at values below fit curve limit, and they will be substituted by the (LLOD * dilution factor) /√2.
Final concentration greater or equal to LLOD * dilution factor will be considered "In range", and all other values, even those substituted, will be identified as below LLOD.

Antibacterial treatment:
Antibacterial treatment will be identified by the sponsor from the Drug name.

Automated-calculated TCC/mL dose cohort:
Automated-calculated cell concentration dose cohorts will be defined by the sponsor based on a new method for the calculation of the total cell concentration and the number of infusion performed:
Cohort 1 - 1st pat.: Patients of cohort 1a
Cohort 2 - 0.6-0.8 x 1: Automated-calculated TCC/mL dose between 0.6 and 0.8 million cells / kg bodyweight and 1 infusion
Cohort 3 - 0.6 x 2: Automated-calculated TCC/mL dose of 0.6 million cells / kg bodyweight and 2 infusions
Cohort 4 – 1.2 x 1: Automated-calculated TCC/mL dose of 1.2 million cells / kg bodyweight and 1 infusion
Cohort 5 – 1.2 x 2: Automated-calculated TCC/mL dose of 1.2 million cells / kg bodyweight and 2 infusions

Naïve and non-naïve to glucocorticosteroids:
Patients naïve and non-naïve to glucocorticosteroids will be identified by the sponsor based on the previous concomitant treatment. Non-naïve patients correspond to patients receiving

glucocorticosteroids before screening and others patients corresponds to naïve patients even if they were treated by glucocorticosteroids after the screening.

Criteria for study treatment discontinuation:
Post-treatment adverse events that will determine transitory or definitive discontinuation of study treatment administration are the following:

Transitory discontinuation:

- Coagulation disorders considered as significant (INR ≥ 2, Fibrinogen < 100 mg/dL, or Platelets < 50.000/mm^3) by the PI prior to each infusion should preclude the administration of HepaStem.
    - For protocol versions 3 and 4: INR ≥ 2, Fibrinogen < 100 mg/dL, or Platelets < 50.000/mm^3
    - For protocol versions 5 and 6: Fibrinogen < 80 mg/dL, or Platelets < 40.000/mm^3
- Absence of portal vein flow prior to the infusion should preclude the administration of HepaStem.
- Mild transfusion-like reaction or other mild adverse events that preclude transitorily the administration of HepaStem.
- Infusions may be restarted after recovery. If planned infusions are not performed within treatment period (+/-2 days), missed infusions will not be given.

Definitive discontinuation:

- Serious and/or severe adverse events assessed as possibly related to HepaStem by the investigator, ie, thrombosis, haemorrhage, anaphylactic shock, severe acute lung injury.
- Other serious and/or severe adverse events not assessed as possibly related to HepaStem but that preclude the continuation of HepaStem infusions in the opinion of the investigator, i.e. severe haemorrhage, septic shock.

The reason of study treatment discontinuation will be documented and the patient will be followed up in the active study period up to Day 28 and thereafter in the long term safety follow-up.

Study withdrawal criteria:

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to major protocol deviation as an inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Patient’s decision to withdraw consent
- Termination of the trial by a regulatory authority or Ethics Committee
- Termination of the trial by the Sponsor
- Termination of trial participation by the Principal Investigator

- Any other reason for withdrawal that the Principal Investigator or patient feels is in the best interest of the patient.

5 Statistical analyses

5.1 Demographic Data

5.1.1 Patient disposition

Main study dates will be presented: first patient screened, first patient included, last patient included, last patient discontinuation. Duration of inclusions and duration of study will be calculated.

The overall number of screen-failure and the overall number of patients included in FAS population and by protocol, SAF population and in PP population will be presented.

The list of patients excluded from these populations (with reason of exclusion) will be given.

The list of patients of the FAS with information on eligibility criteria will be provided.

The list of patients with information on non-inclusion and non-treatment reasons will be presented.

The disposition of patient by country and center will be presented.

The number of patients who discontinued the study prematurely will be summarized in the SAF population. Primary reason for premature discontinuation will also be summarized.
The list of patients who discontinued the study prematurely will also be given with the reason for end of study.
A patient of the SAF is considered as a patient who discontinued the study prematurely if the reason of end of study is not « end of study according to the protocol ».

The number of patients presenting at least one protocol deviation will be given. Protocol deviations as defined during the data review meeting will be listed.

5.1.1.1 Demographic and Baseline characteristics

Patients' demographic (gender, age) will be summarised by planned treatment dose and overall. Descriptive statistics for the following baseline characteristics will be presented:

- Time since ACLF diagnosis (months) for patient included on protocol 1
- Time since the diagnosis of Acute decompensation of Chronic Liver Disease for patient included on protocol 3 only
- Time since admission in current hospital (days)
- Time since admission in current department (days)
- ACLF diagnosed at the time of admission in current hospitalization (Yes/No)
- ACLF diagnosed at the time of admission in current department (Yes/No)

- ACLF grade at the time of diagnosis for patient included on protocol 3 or higher only[1] (Grade 0/Grade 1/Grade 2/Grade 3)

Values at screening and at D1 prior infusion and the change from screening to D1 prior infusion for quantitative parameters or shift table for qualitative parameters will be described for the following parameters:

- ACLF grade
- Scores: West Haven criteria, New MELD score and CLIF-C AD
- Common clinical laboratory tests: WBC, neutrophils, Bilirubin and CRP
- Coagulation parameters: Platelets, INR and Fibrinogen
- Thromboelastogram (central measurements only): Clotting time, Clot Formation Time, Amplitude 10 min, Maximum Clot Firmness and Maximum of Lysis
- Thrombin generation test (central measurements): Lag time, ETP, Peak, Time to peak, Velocity index

The cause of admission in hospital and in current department will be listed.

The following medical history (other than ACLF) will be described:

- cirrhosis history: time since cirrhosis diagnosis (months), etiology of the cirrhosis (alcoholic liver disease, hepatitis C, hepatitis B, non-alcoholic steatohepatitis, genetic disorders, autoimmune disease, other, unknown)
- cardiac, pulmonary, renal, circulatory, coagulation, metabolic/diabetes, neurology, gastrointestinal, oncology/immunology, bleeding and other history: proportion of patient with at least one overall/previous/concomitant medical history, time since diagnosis by categories, Number of pathology / patient by categories
- Overall and concomitant medical history will also be described by SOC and PT.

The specification of the genetic disorders, autoimmune disease and other etiology of the cirrhosis will be listed.

The ACLF medical history will be described in term of:

- Previous episode of Acute Decompensation (Yes/No) for patient included from amendment 3
- Time since last episode of Acute Decompensation (months) for patient included from amendment 3
- Previous episode of ACLF (Yes/No)
- Time since last episode of ACLF (months)
- Factors(s) triggering to the current episode of AD/ACLF: Active alcoholism (Yes/No), Infection (Yes/No), Hepatitis (Yes/No), other (Yes/No), Unknown (Yes/No).

Concomitant treatment will be described by drug class and drug name.

Number of patients with antibacterial concomitant treatment will be provided.

[1] ACLF grade at the time of diagnosis was not collected in the study for the first patients.

The vital signs (height, weight, BMI), the biological analysis (see list below) only at screening will be described in this part:

- Lipase (values and by classes abnormal CS/abnormal NCS/normal)
- Virology status: HBV antigen HBs, HCV antibodies total, HEV antibodies IgG, HEV antibodies IgM, HIV antibodies, Aspergillus (Negative/Positive/Indeterminate/Not done). The values of the positive results will be summarized.
- Pregnancy (Negative/Positive/Not applicable/Not done)
- Urine analysis: Sediment, Creatinin, Glucose, Protein, Albumin (Negative/Positive/Not done)
- Coagulation parameters (values and by classes abnormal CS/abnormal NCS/normal): aPTT(sec), INR, Protein C, Protein S, anti-thrombin III: at screening.

ECG, culture or other fluid culture, transjugular liver biopsy at screening will be described in terms of:

- Results (Normal/Abnormal)

The specification of the abnormality of the results of Imaging-Radiology-ECG analysis will be listed.

All statistical analyses will be based on the safety and per protocol populations and described by dose cohort and overall.

5.2 Efficacy data

5.2.1 Clinical efficacy

All analyses will be provided by planned treatment dose and overall in the SAF and PP populations, unless stated otherwise. The occurrence of liver transplantations will be specified for all tables (footnote) and graphics (symbol or footnote).

Disease scores will be described in terms of:

- West-Haven criteria of HE: values at baseline and each post baseline time point and shift table presenting the grade at baseline versus the worst grade during study will be presented.
- CLIF-OF score and sub-scores with their components: values at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.
- Organ failure (Yes/No) based on CLIF-C OF score: The number and the percentage of patients at baseline and each post-baseline time points will be presented by organ (liver, kidney, cerebral, coagulation, circulatory and respiratory).
- ACLF grade: the number and the percentage of patients at baseline and each post-baseline time points will be presented.

- CLIF-C ACLF score: values at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.
- CLIF-C AD score: values (quantitative and by class: ≥60 /]45-60[/ ≤45) at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.
- MELD scores and number of dialysis in the past week (<2 / ≥2): values at baseline and each post baseline time point and change from baseline at each post baseline time point (not applicable for class description) will be described.
- Child Pugh score: values (quantitative and by class: A / B / C) at baseline and each post baseline time points and change from baseline at each post baseline time point (not applicable for class description) will be described.
- FIB-4 score: values at baseline, D28, M3, M6 and M12 and change from baseline to D28, M3, M6 and M12 will be described.
- APRI scores: values at baseline, D28, M3, M6 and M12 and change from baseline to D28, M3, M6 and M12 will be described.

Graphical representations will be done to represent:

- Individual profiles (overall and by planned cohort dose) of values at baseline and at post-baseline visits and changes from baseline to post-baseline visits will be done only on the SAF population
- Mean evolution (+/- standard deviation) of change from baseline to post-baseline values (by planned treatment group) will also be provided, except for West-Haven criteria.

Evolution from baseline to D28 and M3 will be described only for the patients of the SAF population without liver transplantation at D28 (respectively M3) using paired Hodges & Lehmann estimator with its 95% confidence interval for the new MELD score and the Child-Pugh score.

All previous analyses (tables and graphics), except comparison between visits, for new MELD score and Child-Pugh score will be also performed by automated-calculated cell concentration dose cohorts on the SAF population.

5.2.2 Biological efficacy

All analyses will be provided by planned treatment dose and overall in the SAF and PP populations, unless stated otherwise. The occurrence of liver transplantations will be specified for all tables (footnote) and graphics (symbol or footnote).

Values at baseline and post-baseline visits and changes from baseline to post-baseline visits will be summarized for the following parameters:

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- Common clinical laboratory: WBC, Neutrophil count, Bilirubin, creatinine, albumin, sodium and CRP
- Coagulation parameters, only on SAF population: platelets, INR and aPTT
- Thomboelastogram (central measurements only), only on SAF population: Clotting time and Maximum Clot Firmness
- Thrombin generation test (central measurements), only on SAF population: ETP, Peak and Velocity index

Graphical representations will be done for the previous parameters to represent:

- Individual profiles of values at baseline and at post-baseline visits (only values prior infusion will be presented) and changes from baseline to post-baseline visits (only values prior infusion will be presented) will be done only on the SAF population
- Mean evolution (+/- standard deviation) of change from baseline to post-baseline values (only values prior infusion will be presented) (by planned treatment group) will also be provided.

The same analyses (tables and graphics for individual profiles and mean evolution), but only on the SAF population, will be performed on the short term around infusion with values prior infusion, post-infusion and at the first visit after infusion for the following parameters:

- Coagulation parameters: platelets, INR, aPTT, Fibrinogen and D-dimers
- Thomboelastogram (central measurements only): Clotting time and Maximum Clot Firmness
- Thrombin generation test (central measurements): ETP, Peak and Velocity index

Evolution from baseline to D28 and M3 will be described only for the patients of the SAF population without liver transplantation using paired Hodges & Lehmann estimator with its 95% confidence interval for the following common clinical laboratory parameters:

- WBC, Neutrophil count, Bilirubin, creatinine, albumin, and CRP

All previous analyses (tables and graphics for general overview and/or on the short term around infusion), except comparison between visits, will be also performed by automated-calculated cell concentration dose cohorts on the SAF population for the following parameters:

- Common clinical laboratory: WBC, Neutrophil count, Bilirubin, creatinine, albumin, sodium and CRP
- Coagulation parameters: platelets, INR, aPTT, Fibrinogen (only for short term around infusion) and D-dimers (only for short term around infusion)
- Thomboelastogram (central measurements only), only on SAF population: Clotting time and Maximum Clot Firmness
- Thrombin generation test (central measurements): ETP, Peak and Velocity index

5.2.3 Cytokines analysis

Inflammatory and anti-inflammatory cytokines: at baseline and days 8, 14 and 28 (blood testing in central lab)

All analyses will be provided by planned treatment dose and overall in the SAF and PP populations, unless stated otherwise. The occurrence of liver transplantations will be specified for all tables (footnote) and graphics (symbol or footnote).

Description of each cytokine by class (below LLOD / In range) whatever the visit and only on the SAF population will be provided.

Values at screening and at D1 prior infusion and the change from screening to D1 prior infusion will be summarized:

- Values at screening and at D1 prior infusion and the change from screening to D1 prior infusion for cytokines with at least 80% of values "In range".
- Shift table of values (below LLOD / In range) at screening and at D1 prior infusion for cytokines with a proportion of values "In range" between 20% (greater or equal) and 80% (lesser).

Values at baseline and each post-baseline time point and corresponding changes from baseline to each post-baseline time point will be summarized:

- Quantitative description will be performed only for cytokines with at least 80% of values "in range".
- For other cytokines, a description by class (below LLOD / In range) at each visit will be provided if there are at least 20% of values "in range".

This analyse will be also provided according to the automated-calculated cell concentration dose cohort in the SAF population.

A listing of individual values will also be provided.

5.2.4 Survival analysis

The percentage of mortality, overall survival and transplant free survival will be analysed at D28, M3 and M12 on the SAF population for all patients, for ACLF patient at baseline and for AD patient at baseline.
Moreover a listing of individual data with indicators for death, overall survival and transplant free survival at time points of interest and ACLF grade at screening and baseline will be provided.

5.2.5 Comparison between naïve and non-naïve to glucocorticosteroid groups

Description of values at baseline, D28 and M3 and change from baseline to D28 and M3 for naïve and non-naïves to glucocorticosteroids groups will be provided on the SAF population for the following parameters:

- New score MELD
- Common clinical laboratory: WBC, neutrophils, lymphocytes, Bilirubin, CRP
- Cytokines: CRP (only at baseline and D28)

Difference between naïve and non-naïve to glucocorticosteroids groups will be estimated at baseline, D28 and M3 using a Hodges-Lehmann estimator for independent sample with its 95% confidence interval for the previous parameter.
At D28 and M3, the percentage of mortality, the percentage of overall survival and the percentage of liver transplantation free survival will be also provided for naïve and non-naïve to glucocorticosteroids groups.

5.3 Safety data

5.3.1 Adverse events

All analyses will be provided by planned treatment dose and overall in the SAF population.
The occurrence of liver transplantations will be specified for all tables (footnote).

The number and the percentage of patients having at least the following event and the number of events will be presented at day 28:

- one AE.
- severe AE.
- one SAE.
- one AE/severe AE or SAE related to IMP.
- one AE/severe AE or SAE related to study procedure.
- one AE/severe AE or SAE having led to discontinuation of treatment.
- one SAE having led to death.
- one AE/severe AE or SAE related to IMP having led to discontinuation of treatment.
- one AE/severe AE or SAE related to study procedure having led to discontinuation of treatment.
- one SAE related to IMP having led to death.
- one SAE related to study procedure having led to death.

The same analysis will be also provided according to the automated-calculated cell concentration dose cohort.

The number and the percentage of patients having at least the following event (SAE, AESI) will be presented between day 28 and month 3, and between month 3 and month 12.

- one SAE, only for analysis between day 28 and month 3
- one SAE having led to death
- Liver transplantation
- Outcome of transplantation
- Malignancies
- Hospitalisation for ACLF
- One AE possibly related to IMP

The number and percentage of patients presenting at least one AE will be summarized in a frequency table by body system organ class (SOC) and preferred terms (PT) as per MedDRA dictionary. The number of events will be also summarized in the same table. The table will be displayed by descending order based on the number of patients. Each patient will be counted only once within each classification (SOC / PT).

This analysis will be performed

- by SOC, PT at Day28 for all AE and SAE, between day 28 and month 3 only for AESI and serious AESI, between month 3 and month 12 only for AESI and serious AESI.
- by SOC, PT, and severity at Day28 for all AE, between day 28 and month 3 only for AESI, between month 3 and month 12 only for AESI
- by SOC, PT for AE/AESI related to IMP at Day 28 between day 28 and month 3 and between month 3 and month 12

The same analysis for AE and SAE at Day28, and for all AE/AESI related to IMP will be provided according to the automated-calculated cell concentration dose cohort.

All AEs will be listed separately including at least the following items:

- Dose cohort (1a/1b/2a/2b)
- Patient number
- Age / Sex
- Infusion dates
- The preferred term (PT) and the system organ class (SOC)
- The verbatim
- Date of onset and date of end (study day) or ongoing
- Duration of the AE in days
- Intensity
- Outcome
- Relationship to studied pathology
- Relationship to IMP
- Relationship to specific study procedure
- Actions taken regarding the IMP
- Action taken regarding the event
- Evaluation of Seriousness (SAE onset date, seriousness)

Adverse events related to IMP, serious adverse events, adverse events leading to discontinuation, adverse events leading to death, adverse events of special interest will be listed separately.

5.3.2 Laboratory data

All analyses will be provided in the safety population.
The occurrence of liver transplantations will be specified for all tables (footnote).

The values of the parameters and the corresponding changes from baseline to post-baseline values will be summarized by planned treatment dose and overall at baseline and each post-baseline time point.

The status of the results (normal/abnormal not CS / abnormal CS) will be summarized by dose cohort and overall at baseline and each post-baseline time point and for each parameter. Shift tables presenting the status at baseline versus the worst status during study will be presented by planned treatment dose and overall.

The following parameters will be described:

- Common clinical laboratory tests[1] at baseline, days 4, 8, 12, 14, 21, 28, and months 2, 3, 6 and 12.
    - White Blood Cell count, hemoglobin, hematocrit, neutrophils, lymphocytes, monocytes, eosinophils, basophils.
    - platelets (additional measures on infusion day: post infusion at 4h, 24h, 48h, and 72h at day 1, and day 8 in cohort 2b and 2d),
    - GOT, GPT, bilirubin, alkaline phosphatase, γ GT,
    - Creatinine, Urea or BUN
    - CRP
    - INR, aPTT (in sec)
    - Serum albumin, sodium, potassium,
    - Blood gas: PaO2, SpO2 and FiO2

- Coagulation parameter[1]: at baseline, and day 8 and additional measures on infusion day (post infusion at 4h, 8h, 12h, 24h, 48h and 72h at day 1, and day 8 in cohort 2b and 2d).
    - INR
    - aPTT (in sec)
    - fibrinogen
    - D-Dimers

[1] For first patients some parameters were not collected in the study

- Coagulation factors for both intrinsic (factors VIII, IX, XI, XII) and extrinsic pathway (factors II, V, VII, X): at baseline and day 8 and additional measures on infusion day (24h post infusion at day 1, and day 8 in cohort 2b).

- Thomboelastogram (central measurements only): at baseline and days 4, 8 12, 14, 21 and 28, and additional measures on infusion day (4h post infusion at day 1, and day 8 in cohort 2b and 2d) (blood testing in central lab):
    - Clotting time
    - Clot Formation Time
    - Amplitude 10 min
    - Maximum Clot Firmness
    - Maximum of Lysis

- Thrombin generation test (central measurements): at baseline and days 4, 8 12, 14, 21 and 28, and additional measures on infusion day (4h post infusion at day 1, and day 8 in cohort 2b and 2d) (blood testing in central lab):
    - Lag time
    - ETP
    - Peak
    - Time to peak
    - Velocity index

- Anti-HLA antibodies (class I and class II by luminex method): at baseline, day 28, and month 3, 6 and 12 (blood testing in central lab)
- Abdominal and portal system ultrasonography and Doppler: at baseline, days 4, 8, 12, 14 and 28 and month 12

- Chest X Ray and Cardiac US Doppler: at baseline and month 12:
  For Cardiac US Doppler only: If abnormal, describe (left-right shunt/Right-left Shunt/No shunt), Pulmonary arterial pressure.

At Minima, for each patient, parameters results, status, and specification of abnormalities will be listed.
"Patient fasting ? (yes/no) and Samples according to study manual (yes/no) ?" will be described for central laboratory measurement in listing only.
The values of laboratory parameters will be summarized by standard unit as defined in SDTM database except for CRP of common clinical laboratory test summarized by mg/L.

For platelets and coagulation parameters the status of the results (normal/abnormal not CS / abnormal CS) and shift tables of the status will be also presented according to the automated-calculated cell concentration dose cohort.
Anti-HLA antibodies analysis will be also provided according to the automated-calculated cell concentration dose cohort.

5.3.3 Physical examination

All analyses will be provided by planned treatment dose and overall in the safety population.
The occurrence of liver transplantations will be specified for all tables (footnote).

The status of the results (normal /abnormal) of Physical examination (cardiac, pulmonary, abdomen, upper extremities, lower extremities, neurologic, skin, other) will be summarized by treatment dose and overall and baseline and at each post-baseline time point. Additionally, shift tables presenting the status at baseline versus the worst status during study will be presented by treatment dose and overall.

Physical examination will be described at baseline, days 4, 8, 12, 14, 21 and 28 and months 2, 3, 6 and 12.

At minima, for each patient, parameters results, status, and specification of abnormalities will be listed.

5.3.4 Vital signs

The values of the parameters and the corresponding changes from baseline to post-baseline values will be summarized by planned treatment dose and overall at baseline and each post-baseline time point in the safety population.
The occurrence of liver transplantations will be specified for all tables (footnote).

Vital signs will be described at baseline, days 1, 4, 8, 12, 14, 21 and 28 and months 2, 3, 6 and 12, and additional measurements on infusion day (during and after infusion at day 1, and day 8 in cohort 2b).

At minima, for each patient, parameters results, status, and specification of abnormalities will be listed.

The following parameters will be described: Body Temperature, Respiratory Rate, Heart Rate, Arterial Pressure, Pulse Oxymetric Saturation.

5.3.5 IMP administration

The following information will be described by treatment group and overall in the safety population:

- Proportion of patient who completed all planned infusions
- Number of patients who performed each infusion for patients with an administration scheme with repeated infusions
- Duration of IMP exposure: expressed in days and calculated as

  (Date of last IMP infusion) - (date of first IMP infusion) +1
- Total dosage of HepaStem
- For each infusion:

- Time from Hydrocortisone administration (minutes)
- Total theoretical dose of HepaStem
- Total effective dose (actually infused) of HepaStem
- Effective (actual) dose of HepaStem infused per kg.
- Intravenous Access (Peripheral/Central)
- Duration of infusion (min)
- Minimum Flow rate during infusion (mL/min)
- Maximum Flow rate during infusion (mL/min)
- Interruption of the flow rate of HepaStem (Yes/No)
- Actual volume of HepaStem infused (mL)
- Infused Volume ratio

The occurrence of liver transplantations will be specified for all tables (footnote).

5.4 Analysis of sub-groups/complementary analysis

All analysis will be performed by planned treatment dose and overall.

6 Tables, Listings and Graphs (TLG)

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ÉTUDE HEP101

ÉTUDE CLINIQUE MULTICENTRIQUE DE PHASE II VISANT A EVALUER LA SECURITE ET L'EFFICACITE PRELIMINAIRE DE 2 DOSES D'HEPASTEM CHEZ DES PATIENTS SOUFFRANT D'INSUFFISANCE HEPATIQUE AIGUË SUR UNE MALADIE CHRONIQUE DU FOIE.

Version 1.1 du protocole (28 juin 2016)

Formulaire d'information pour le patient

Ce formulaire d'information pour le patient et le formulaire de consentement éclairé sont destinés aux patients souffrant d'une insuffisance hépatique aiguë sur maladie chronique du foie (ACLF) qui sont invités à participer à une étude de recherche clinique réalisée avec HepaStem

Promoteur de l'étude : **Promethera Biosciences**
Adresse : **Rue Granbonpré 11**
1435 Mont St Guibert
Belgique

Investigateur principal : _____
Adresse : _____
Numéro de téléphone : _____

Introduction

Vous êtes invité(e) à participer à une étude clinique destinée à évaluer un médicament expérimental pour le traitement de votre maladie. Un médicament expérimental est un médicament faisant encore l'objet d'études pour évaluer son efficacité, sa sécurité d'emploi ou son mécanisme d'action.

L'investigateur (ou médecin de l'étude, c.-à-d. le médecin responsable de cette étude de recherche à l'hôpital) et le promoteur (la firme qui initie et finance cette recherche) espèrent que ce médicament expérimental peut présenter des avantages pour le traitement de patients atteints de la même maladie que la vôtre. Néanmoins, rien ne garantit que vous tiriez un bénéfice de votre participation à cette étude.

Avant que vous n'acceptiez de participer à cette étude, nous vous invitons à prendre connaissance de ses implications en termes d'organisation, risques et bénéfices éventuels, afin que vous puissiez prendre une décision en toute connaissance de cause. C'est ce qu'on appelle donner un « consentement éclairé ».

Veuillez lire attentivement ces quelques pages d'information et poser toutes les questions que vous souhaitez à l'investigateur ou à la personne qui le représente. Ce document comprend 2 parties : l'information essentielle à votre prise de décision et votre formulaire de consentement écrit.

Avant de prendre une décision, vous devez savoir que :

- Cette étude clinique est mise en œuvre après avoir fait l'objet d'une évaluation par un comité d'éthique (de Louvain) après consultation des comités d'éthique d'autres centres participants.
- Votre participation est volontaire et doit rester libre de toute contrainte. Elle nécessite la signature d'un document exprimant votre consentement. Même après avoir signé ce document, vous pouvez interrompre votre participation en informant le médecin investigateur.

Votre décision de ne pas ou de ne plus participer à l'étude n'aura aucun impact sur la qualité de vos soins ni sur vos relations avec le médecin investigateur.

- Les données recueillies au cours de l'étude sont confidentielles. Votre anonymat sera toujours garanti, y compris lors de la publication des résultats.
- Le promoteur a souscrit une assurance au cas où vous subiriez un dommage lié à votre participation à cette étude clinique.

  Compagnie d'assurances : QBE Syndicate
  Numéro de la police : 16ME305419EA
- Votre participation à cette étude est totalement gratuite. Aucun frais ne vous sera facturé pour les examens spécifiques liés à cette étude. Cependant, vous ne serez pas rémunéré(e) pour votre participation à cette étude.
- Les dépenses liées à votre transport jusqu'à l'hôpital (comme les frais de voiture, de taxi, le ticket de train, etc.) vous seront remboursées avec des vouchers (1 pièce/visite de l'étude effectuée après votre sortie de l'hôpital). Veuillez contacter le personnel de l'étude pour les détails pratiques.
- Le promoteur remboursera l'hôpital/médecin investigateur pour tous les frais de visites/consultations, examens et traitements spécifiques à l'étude.
- Vous pouvez contacter le médecin investigateur ou un membre de son équipe à tout moment si vous avez besoin d'informations complémentaires.

Objectifs et description du protocole de l'étude

Vous êtes invité(e) à participer à une étude clinique portant sur le traitement par des cellules de foie humain (appelées « cellules progénitrices ») provenant de foies de donneurs. On espère que ces cellules ont un effet combiné systémique (dans tout le corps) et local dans le foie. Nous pensons qu'elles jouent un rôle immunomodulateur, c.-à-d. qu'elles aident à réguler la réponse inflammatoire exagérée qui est observée au cours de votre maladie, en particulier qu'elles aident à résoudre l'épisode aigu actuel de votre affection. Les cellules testées sont appelées « cellules progénitrices allogéniques (c.-à-d. provenant d'une autre personne) isolées à partir de foie humain adulte » ou HepaStem.
Les cellules sont des cellules souches isolées à partir de foies adultes et ne sont pas des cellules souches embryonnaires. Elles sont conservées selon une méthode spécifique et les autorités de santé ont approuvé leur utilisation chez des patients dans le cadre d'une étude clinique.

Douze (12) patients seront inclus dans cette étude en Europe, dont environ 6 en Belgique. Le médecin investigateur proposera à des patients qui, comme vous, présentent un diagnostic de cirrhose et une insuffisance hépatique aiguë sur maladie chronique du foie, de participer à cette étude.

L'objectif principal de cette étude est d'évaluer la sécurité et l'efficacité de deux (2) posologies d'HepaStem administrées 4 fois sur une période de 14 jours (1 administration tous les 3 jours).

Il s'agit d'une étude réalisée en ouvert pour évaluer 2 schémas posologiques, ce qui signifie que vous recevrez

- 4 administrations de la dose faible de cellules (250 millions de cellules pour chaque administration) sur une période de 14 jours (cela signifie l'administration d'1 seringue de 50 ml deux fois par semaine pendant 2 semaines), ou

- 4 administrations de la dose élevée de cellules (500 millions de cellules pour chaque administration) sur une période de 14 jours (cela signifie l'administration de 2 seringues de 50 ml deux fois par semaine pendant 2 semaines).

Dans les deux cas, vous et votre médecin investigateur saurez quelle posologie vous recevez.

Déroulement de l'étude

Après la phase de traitement de 2 semaines par les cellules et une période supplémentaire de 2 semaines de surveillance, vous resterez encore dans l'étude pendant 1 an (phase de suivi). Au cours du premier mois, vous ferez l'objet de 8 évaluations d'étude, c.-à-d. que vous subirez ces jours-là des procédures et des examens spécifiques à l'étude. Pendant la phase de suivi, vous ferez l'objet de 4 évaluations d'étude.

Les examens et procédures nécessaires liés à l'étude sont décrits ci-dessous. Certains de ces examens et procédures font partie des soins standards prodigués par votre hôpital, tandis que d'autres sont offerts dans le cadre de cette étude.

Votre participation à l'étude sera constituée de 3 phases :

1 Phase de sélection : pendant cette phase, votre médecin investigateur vérifiera si vous êtes éligible pour participer à cette étude, et établira un relevé de vos antécédents médicaux et des résultats de certains examens réalisés après avoir reçu votre accord pour participer à l'étude.

Tout au plus 25 ml supplémentaires de sang (environ 2 cuillères à soupe) seront prélevés. Des prises de sang sont déjà réalisées dans le cadre des soins liés à votre situation clinique actuelle ; aucune piqûre supplémentaire ne sera donc réalisée sur votre bras pour prélever ces tubes de sang.
Certaines analyses d'urine seront également réalisées.
Les échantillons de sang et d'urine permettront d'analyser des paramètres qui fourniront des informations sur l'état de vos organes (foie, reins, sang), sur votre statut virologique (notamment pour l'hépatite et l'infection à VIH) et sur votre immunité.
Si ce n'est pas déjà fait, on réalisera une échographie de votre foie et de votre cœur, une radiographie du thorax et un électrocardiogramme. Ces examens ne sont pas invasifs et ils sont indolores.
Si vous avez déjà subi une biopsie du foie pendant cette hospitalisation, les résultats de cet examen seront collectés.
Vous recevrez une « carte d'urgence » mentionnant toutes les informations sur l'étude. Veuillez porter cette carte sur vous en permanence. Si vous n'êtes pas hospitalisé(e), veuillez montrer cette carte à votre médecin et l'informer que vous participez à cette étude.

Après la phase de sélection, si vous répondez à tous les critères nécessaires pour entrer dans l'étude, vous serez en mesure de débuter la phase active de l'étude et vous recevrez le médicament HepaStem.

2 Phase active de l'étude : Pendant cette phase, vous recevrez 4 administrations d'HepaStem sur une période de 14 jours. En fonction du groupe auquel vous appartenez, vous recevrez 250 ou 500 millions de cellules par administration, ce qui équivaut à un volume de 50 ou 100 ml de liquide.

HepaStem sera administré par voie intraveineuse, ce qui signifie qu'une aiguille sera introduite dans une veine de petit ou grand calibre pour administrer le médicament. En vue de prévenir les réactions indésirables de votre corps au médicament, un médicament sera administré avant la perfusion d'HepaStem.

Pendant chaque administration et dans tous les cas, 2, 3 et 4 semaines après la 1ère administration, vous ferez l'objet d'examens cliniques et des échantillons de sang seront prélevés (environ 15 mL de sang soit 1 cuillère à soupe). Les échantillons de sang permettront d'obtenir des informations sur l'état de vos organes (foie, reins, sang).
Une échographie de votre cœur sera réalisée après la 1ère administration et une échographie de votre foie sera réalisée après avant chaque perfusion, puis 2 et 4 semaines après la 1ère administration.

3 Phase de suivi : pendant cette période, vous serez invité(e) à revenir à l'hôpital 2 mois, 3 mois, 6 mois et 12 mois après l'administration de la 1ère perfusion de cellules. Au cours de ces visites, vous ferez l'objet d'un examen clinique et un échantillon de sang sera prélevé (environ 15 mL de sang soit 1 cuillère à soupe). Les résultats de ces examens seront collectés.
Les échantillons de sang permettront d'analyser des paramètres qui fourniront des informations sur l'état de vos organes (foie, reins, sang).
Pendant cette période, si vous avez subi une transplantation du foie, un échantillon de votre foie explanté sera collecté si possible.

Après la phase de suivi, nous vous demanderons si vous acceptez d'être inscrit(e) dans notre registre dans le cadre d'un suivi régulier et complémentaire.

Risques et inconvénients

A : Effets secondaires du médicament de l'étude

- Tous les médicaments ont des effets secondaires connus ou imprévisibles. Même si l'étude précédente a révélé qu'HepaStem était bien toléré, il est toujours possible que vous présentiez les effets secondaires suivants : **à court terme :**
  - Thrombose
- Affection respiratoire Réaction d'hypersensibilité ou réaction à la perfusion : ceci se produit lorsque le système immunitaire du corps sur-réagit à quelque chose comme un médicament. La réaction d'hypersensibilité peut comprendre : irritation de la peau, rougeur, démangeaisons, gonflement, suintement, formation de croûtes, éruptions, toux ou essoufflement, enrouement de la voix, maux de tête, nez bouché ou qui coule, éternuements, yeux rouges, douleurs à l'estomac, nausée, vomissements, diarrhée, fatigue, mal de gorge, vertiges. Ces réactions peuvent être préjudiciables, inconfortables ou occasionnellement, fatales (en cas d'anaphylaxie). **à moyen ou long terme :**
  - distribution dans différents organes où les cellules peuvent favoriser le développement d'une tumeur, même si ces effets ont été rarement rapportés avec la thérapie cellulaire réaction immunitaire car HepaStem est constitué de cellules provenant d'une autre personne, ce qui peut éventuellement induire une réaction de rejet des cellules.

D'autres risques et inconvénients inconnus à ce jour pourraient également apparaitre. Il est donc très important de signaler rapidement tout nouveau problème de santé au médecin investigateur, que vous pensiez ou non qu'il soit en rapport avec l'étude.

C : Risques associés aux procédures spécifiques à l'étude

Il existe également des risques/inconvénients associés aux examens spécifiques qui seront réalisés dans le cadre de cette étude :

- L'administration intraveineuse peut provoquer les effets suivants :
    - Douleur, comme pour toute injection.
    - Infection. Toute ouverture au niveau de la peau peut comporter un risque d'infection, même si l'insertion intraveineuse est une procédure aseptique.
    - Phlébite : inflammation d'une veine pouvant être causée par une infection.
    - Infiltration : une infiltration survient lorsqu'un liquide ou un médicament administré par voie intraveineuse pénètre accidentellement dans le tissu environnant plutôt que dans la veine.
    - Embolie : un caillot sanguin ou une autre particule solide, ou encore une bulle d'air, peut être introduit dans la circulation au cours de l'administration IV et bloquer finalement un vaisseau. Cependant, il est presque impossible d'injecter de l'air au cours d'une administration IV par voie périphérique. Le risque est plus élevé en cas d'administration IV par voie centrale.
- La **prise de sang** nécessaire aux analyses peut (rarement) causer une douleur, un saignement, une contusion (bleu) ou une infection autour du site d'injection. Chez certains patients, des étourdissements, ou même un évanouissement, peuvent survenir pendant la procédure.

L'équipe qui réalisera la prise de sang fera usage des meilleurs soins médicaux pour empêcher ou réduire au minimum ces inconforts.

Notification de nouvelles informations

Il est possible que, pendant le déroulement de l'étude clinique, de nouvelles informations importantes sur HepaStem, le médicament étudié, deviennent disponibles. Vous serez informé(e) au moment opportun de tout élément nouveau susceptible de modifier votre décision de poursuivre votre participation à cette étude.

Si, au vu de ces nouvelles informations, vous décidez d'interrompre votre participation à l'étude, votre médecin investigateur veillera à ce que vous continuiez à recevoir le meilleur traitement possible.

Contraception, grossesse et allaitement

Participant de sexe féminin : Étant donné que les effets d'HepaStem sur un enfant à naître ou un nourrisson ne sont pas parfaitement connus, vous ne serez pas autorisée à participer à cette étude clinique si vous êtes enceinte, si vous souhaitez tomber enceinte ou si vous allaitez.

Si vous choisissez de participer à cette étude, vous devrez utiliser l'une des méthodes contraceptives autorisées (de manière à ne pas tomber enceinte). Votre médecin discutera avec vous des différentes options adéquates.

Bénéfices

Si vous acceptez de participer à cette étude, HepaStem pourra ou non s'avérer bénéfique pour le traitement de la maladie dont vous êtes atteint(e) ou soulager vos symptômes, en particulier pour aider à résoudre la décompensation aiguë actuelle de la fonction de votre foie.

Les informations obtenues grâce à cette étude peuvent contribuer à une meilleure connaissance de l'utilisation de ce médicament ou au développement d'un nouveau médicament pour le traitement de l'insuffisance hépatique aiguë sur maladie chronique du foie chez de futurs patients.

Traitement alternatif :

À ce jour, aucun traitement de votre affection approuvé par les autorités n'est disponible en Europe.

Le seul traitement actuellement disponible est la greffe de foie. Cependant, ce traitement est loin d'être idéal vu le manque d'organes pour la transplantation.

Retrait de l'étude

Votre participation est volontaire et vous avez le droit de vous retirer de l'étude à tout moment et sans devoir vous justifier. Néanmoins, il peut être utile pour le médecin investigateur et pour le promoteur de l'étude de savoir si vous vous retirez de l'étude car les contraintes liées au traitement sont trop importantes (par exemple, trop d'effets secondaires désagréables).

Il est aussi possible que le médecin investigateur vous retire de l'étude car vous êtes enceinte, car il/elle pense que c'est mieux pour votre santé ou car il/elle constate que vous ne respectez pas les consignes données aux participants.

Enfin, il est également possible que les autorités compétentes nationales ou internationales, le comité d'éthique qui a initialement approuvé l'étude ou le promoteur, décident d'interrompre l'étude.

Si vous retirez votre consentement à participer à l'étude, afin de garantir la validité de la recherche, les données encodées jusqu'au moment du retrait, seront conservées. Aucune nouvelle donnée ne sera envoyée au promoteur.

Si vous retirez votre consentement à participer à l'étude, vous pouvez contacter l'investigateur et demander que vos échantillons qui n'ont pas encore été utilisés, soient détruits. Les résultats obtenus de vos échantillons avant le retrait de votre consentement, restent la propriété du promoteur de l'étude.

Traitement après l'arrêt de l'étude

Dans toutes ces situations de retrait de l'étude mais également lorsque la période de participation prévue est arrivée à son terme, votre médecin investigateur évaluera votre état de santé et vous prescrira le meilleur traitement disponible.

Échantillons de matériel biologique collectés au cours de l'étude

Le promoteur de l'étude s'engage à ce que les échantillons (p. ex. sang, urine, tissu hépatique) ne soient utilisés que dans le cadre de l'étude.

- La procédure de codage des échantillons est la même que celle utilisée pour vos données médicales. Les échantillons envoyés au promoteur ne comprendront donc que votre code ID de l'étude.

- Le gestionnaire de ces échantillons (laboratoire d'hématologie des Cliniques St Luc, Bruxelles et Translational Research Center, KU Leuven, Louvain) s'engagent à les utiliser dans le contexte de la recherche clinique et de les détruire à la fin de la période de stockage prévue.

- L'échantillon de matière biologique prélevé est considéré comme un « don » et vous devez savoir que, en principe, vous ne recevrez aucun avantage financier (royalties) associé au développement d'un nouveau traitement dérivé de l'utilisation de votre don de matière biologique, qui pourrait présenter une valeur commerciale.

Le surplus de vos échantillons sera détruit dès que les analyses décrites dans ce document auront été réalisées (au plus tard, un an après la fin de l'étude).

Confidentialité et protection des données

Votre participation à l'étude signifie que vous consentez à la collecte de données à votre sujet par l'investigateur et à l'utilisation de ces données par le promoteur à des fins de recherches et en lien avec des publications scientifiques et médicales.
Vous avez le droit de demander à l'investigateur quelles données sont collectées à votre sujet et quelle est leur utilisation dans le cadre de l'étude. Ces données concernent votre état clinique actuel, mais également une partie de vos antécédents, les résultats des examens réalisés dans le contexte de votre prise en charge selon les standards actuels et évidemment, les résultats des examens requis par le protocole. Vous avez le droit de consulter ces données et de les corriger si elles sont incorrectes. Ces droits sont garantis par la loi du 8 décembre 1992 relative à la protection de la vie

privée à l'égard du traitement de données à caractère personnel et par la loi du 22 août 2002 relative aux droits du patient.
L'investigateur a un devoir de confidentialité vis-à-vis des données collectées.
Cela signifie qu'il/elle s'engage, non seulement à ne jamais révéler votre nom dans le contexte d'une publication ou d'une conférence, mais également qu'il/elle codera (votre identité sera remplacée par un code ID dans l'étude) vos données avant de les envoyer au gestionnaire de la banque des données collectées (Clinical Department, Promethera Biosciences).
L'investigateur et son équipe seront donc les seules personnes capables d'établir un lien entre les données transmises au cours de l'étude et votre dossier médical. Pour la présente étude, la loi exige que ce lien avec votre dossier soit conservé pendant minimum 30 ans et maximum 50 ans, loi belge du 19 décembre 2008 relative à l'obtention de matériel biologique humain et les arrêtés royaux qui s'y rapportent.
Les données personnelles transmises ne contiendront aucune combinaison d'éléments qui pourraient permettre de vous identifier.
Pour le gestionnaire des données de l'étude désigné par le promoteur, les données transmises ne permettront pas de vous identifier. Ce dernier est responsable de la collecte des données recueillies par tous les investigateurs participant à l'étude, leur traitement et leur protection selon les exigences de la loi belge relative à la protection de la vie privée.
Afin de vérifier la qualité de l'étude, il est possible que vos dossiers médicaux soient examinés par des personnes soumises au secret professionnel et désignées par le comité d'éthique, le promoteur de l'étude ou un audit indépendant. Dans tous les cas, l'examen de votre dossier médical sera sous l'entière responsabilité de l'investigateur et sous la supervision de l'un de ses collaborateurs, qu'il/elle aura désigné.
Les données de l'étude (codées) pourront être envoyées à des autorités de règlementation belges ou autres, aux comités d'éthique pertinents, à d'autres médecins et/ou à des organisations travaillant en collaboration avec le promoteur.
Elles pourront également être envoyées vers d'autres sites du promoteur en Belgique et dans d'autres pays où les standards en termes de protection des données personnelles peuvent être différents ou moins rigoureux. Comme indiqué ci-dessus, les données transmises sont codées. Le promoteur s'engage donc au respect des contraintes de la directive européenne et de la législation belge sur la protection de la vie privée.
Votre consentement à participer à la présente étude implique par conséquent également, votre consentement à l'utilisation de vos données médicales codées pour les objets décrits dans le présent feuillet d'information et à leur transmission aux personnes et autorités mentionnées ci-dessus.
Le promoteur s'engage à n'utiliser les données collectées que dans le contexte de l'étude à laquelle vous participez.

Assurance

Toute participation à une étude clinique comprend des risques, aussi faibles soient-ils. Même si aucune erreur n'a été commise, le promoteur assume la responsabilité de tout dommage causé au participant (ou dans le cas d'un décès, ses ayants-droits) et lié directement ou indirectement à sa participation à l'étude. Le promoteur a souscrit une assurance couvrant cette responsabilité.
Il vous est donc demandé d'informer l'investigateur de tout nouveau problème de santé avant de consulter un autre médecin, de prendre un autre médicament ou de recevoir tout autre traitement médical. Si pour une raison quelconque, vous consultez un autre médecin pendant la présente étude clinique, vous devez l'informer que vous participez à une étude clinique/lui présenter votre carte de participant à une étude clinique. Ceci peut être important pour établir un diagnostic et traiter vos plaintes.

Si l'investigateur pense d'un lien avec l'étude est possible (l'assurance ne couvre pas la progression naturelle de votre maladie ou les effets secondaires connus de votre traitement normal), il/elle en informera le promoteur de l'étude, qui initiera la procédure de déclaration à la compagnie d'assurance. Cette dernière nommera un expert – si elle le considère nécessaire – pour établir s'il existe un lien entre votre nouveau problème de santé et l'étude.
Dans le cas d'un désaccord avec l'investigateur ou l'expert désigné par la compagnie d'assurance, et également si vous en ressentez la nécessité, vous ou – en cas de décès - vos ayants-droits pouvez entamer une procédure à l'égard de l'assureur, directement en Belgique (nom de la compagnie d'assurance, numéro de la police, contact).
La loi assure que l'assureur peut être convoqué par le juge de l'endroit où l'événement donnant lieu au dommage s'est déroulé, ou devant le juge de votre domicile, ou devant le juge du siège social de l'assureur.

Si vous participez à cette étude clinique, nous vous demandons :

- De ne pas boire d'alcool pendant au moins les 5 semaines suivant le début de votre participation.
- De collaborer pleinement au bon déroulement de cette étude.
- De ne dissimuler aucune information relative à votre état de santé, aux médicaments que vous prenez ou aux symptômes que vous présentez.
- De ne participer à aucune autre étude clinique impliquant un traitement expérimental (médicament, dispositif médical ou procédure) pendant votre participation à cette étude.
- De porter en permanence sur vous la « carte d'urgence ». Cette précaution est indispensable pour garantir votre sécurité si vous deviez recevoir des soins d'urgence dans une institution qui ne vous connaît pas.

Contact

Si vous avez besoin d'informations supplémentaires, mais aussi en cas de problème ou d'inquiétude, vous pouvez contacter le médecin investigateur ……. ou un membre de son équipe de recherche ……. au numéro de téléphone suivant (xx / xxx-xx-yy) ……..

En cas d'urgence ou en-dehors des heures de consultation, vous pouvez contacter le médiateur…… au numéro de téléphone suivant ……..., en mentionnant que vous participer à une étude clinique. Votre dossier contiendra les informations sur cette étude clinique, qui seront utiles pour le médecin de garde.

ÉTUDE HEP101

Étude Clinique multicentrique de phase II visant à évaluer la sécurité et l'efficacité préliminaire de 2 doses d'HepaStem chez des patients souffrant d'insuffisance hépatique aiguë sur une maladie chronique du foie.

Consentement éclairé du patient

ID du patient de l'étude : ……..

Participant

Je déclare que j'ai été informé(e) sur la nature de l'étude, son but, sa durée, les éventuels bénéfices et risques et ce que l'on attend de moi. J'ai pris connaissance du document d'information. J'ai eu suffisamment de temps pour y réfléchir et en parler avec une personne de mon choix, p. ex. mon médecin généraliste (MG) ou un membre de ma famille.

J'ai eu l'occasion de poser au médecin investigateur toutes les questions qui me sont venues à l'esprit et j'ai obtenu une réponse satisfaisante à mes questions.

Je comprends que ma participation à cette étude est volontaire et que je suis libre de mettre fin à ma participation à cette étude sans que cela ne modifie mes relations avec l'équipe thérapeutique en charge de ma santé.

Je comprends qu'au cours de cette étude clinique, mes données personnelles (en particulier mon dossier médical) seront récoltées, conservées et analysées. L'utilisation des informations relatives à ma santé est conforme aux dispositions légales et nécessite un consentement éclairé et volontaire préalable à ma participation à cette étude. Sans avoir signé le formulaire de consentement, je ne peux pas participer à l'étude.
Les données personnelles sont codées. J'ai le droit de consulter/corriger ces données.

Le consentement à la collecte et au traitement de mes données personnelles, en particulier des informations relatives à ma santé, est irrévocable. J'ai déjà été informé(e) que je peux mettre un terme à ma participation à cette étude à tout moment. Dans le cas où je retire mon consentement, je consens à ce que les données collectées jusqu'à ce moment-là puissent être analysées de manière pseudonyme (codées)

J'accepte que mes données soient conservées après la fin de l'étude ou mon interruption de l'étude, conformément aux exigences nationales requises.

J'accepte que les données d'étude collectées en vue d'atteindre les objectifs de cette étude puissent être traitées ultérieurement, pour autant que ce traitement soit limité au cadre de la présente étude pour une meilleure connaissance de la maladie et de son traitement.

J'accepte que le promoteur conserve des échantillons de matériel biologique récoltés en cours de l'étude après la fin de l'étude clinique, à des fins de recherches ultérieures mais limitées au cadre de la présente étude.

J'accepte que mon MG ou d'autres spécialistes en charge de ma santé soient informés de ma participation à cette étude clinique.

J'ai reçu une copie du document d'information du participant et du formulaire de consentement éclairé.

Prénom du patient : ……Nom du patient : ……

Signature : …… Date : ……

Témoin/interprète

J'ai été présent pendant l'entièreté du processus d'information du patient et je confirme que les informations relatives aux objectifs et procédures de l'étude ont été fournies de manière adéquate, que le participant (ou son représentant légal) a apparemment compris l'étude et que le consentement à participer à l'étude a été donné librement.

Prénom et nom du témoin/de l'interprète : ……..

Signature : …… Date : ……

Médecin investigateur

Je soussigné(e), ……., médecin investigateur, confirme avoir fourni verbalement les informations nécessaires sur l'étude et avoir fourni une copie du document d'information au participant.

Je confirme qu'aucune pression n'a été exercée pour persuader le patient d'accepter de participer à l'étude et que je suis prêt(e) à répondre à toutes les questions supplémentaires si cela s'avère nécessaire.

Je confirme travailler en accord avec les principes éthiques énoncés dans la dernière version de la « Déclaration d'Helsinki », des « Bonnes pratiques Cliniques » et de la loi nationale en vigueur relative aux expérimentations sur la personne humaine.

Prénom du médecin investigateur : ……Nom du médecin investigateur : ……

Signature : …… Date : ……

HEP101 STUDIE
MULTICENTRALE FASE II VEILIGHEIDSSTUDIE EN EFFECTIVITEIT-VOORSTUDIE VAN 2 DOSERINGSREGIMES VAN HEPASTEM BIJ PATIËNTEN MET ACUUT-OP-CHRONISCHE LEVERFALEN
Protocol Versie 1.1_BE (28 juni 2016)

Patiënteninformatie

Deze patiënteninformatie en dit toestemmingsformulier na informatie zijn bedoeld voor patiënten die lijden aan acuut-op-chronisch leverfalen die worden uitgenodigd om aan een wetenschappelijk onderzoek met HepaStem deel te nemen

Studiesponsor: **Promethera Biosciences**
Adres: **Rue Granbonpré 11**
1435 Mont St Guibert
België

Voornaamste onderzoeker: _____
Adres _____
Telefoonnummer: _____

Introductie

U wordt uitgenodigd om deel te nemen aan een klinische studie met het oog op de evaluatie van een medisch onderzoeksproduct voor de behandeling van uw ziekte. Een medisch onderzoeksproduct is een medisch product dat nog steeds wordt bestudeerd om de effectiviteit, veiligheid en werking ervan te evalueren.

De Onderzoeker (of de studiearts is de arts die verantwoordelijk is voor deze wetenschappelijke studie in het ziekenhuis) en de Sponsor (bedrijf dat het wetenschappelijk onderzoek start en financiert) hopen dat dit medisch product helpt bij de behandeling van patiënten met dezelfde ziekte als de uwe. Er is echter geen waarborg dat uw deelname aan deze studie uw gezondheid ten goede komt.

Vooraleer u instemt om aan deze studie deel te nemen, vragen we u om rekening te houden met de implicaties op het vlak van organisatie, mogelijke risico's en voordelen, wat u in staat moet stellen om, volledig bewust van de implicaties, een beslissing te nemen. Dit is gekend als het geven van een "geïnformeerde toestemming".

Gelieve deze informatie nauwkeurig te lezen en leg alle vragen waarmee u zit voor aan de onderzoeker of zijn/haar vertegenwoordiger. Dit document bestaat uit 2 delen: de informatie die essentieel is voor uw beslissing en, het ingevulde en ondertekende toestemmingsformulier.

Vooraleer u beslist, moet u bewust zijn van het volgende:

- Deze klinische studie wordt uitgevoerd nadat ze door een ethisch comité (in Leuven) werd beoordeeld na overleg met de ethische comités van andere deelnemende centra.
- Uw deelname is vrijwillig en moet verder zonder enige dwang verlopen. Een ondertekening van een document waarin u uw toestemming te kennen geeft, is vereist. Zelfs nadat u dit

document hebt ondertekend, kunt u uw deelname stopzetten door de onderzoeker hierover te informeren. Uw beslissing om niet deel te nemen zal geen impact hebben op de kwaliteit van de zorg of op uw relatie met de onderzoeker.
- De gegevens die in deze studie worden verzameld zijn vertrouwelijk en uw anonimiteit is altijd gewaarborgd, ook tijdens de publicatie van de resultaten.
- De sponsor heeft een polis getekend bij een verzekeringsmaatschappij, ingeval u enige schade zou oplopen in verband met uw deelname aan deze studie.

  Verzekeringsmaatschappij: QBE Syndicate

  Polisnummer: 16ME305419EA
- Uw deelname aan de studie is volledig gratis. U zult geen kosten aangerekend krijgen voor de onderzoeken die specifiek zijn voor deze studie. U zult echter ook niet betaald worden voor uw deelname aan deze studie.
- Uw onkosten voor uw vervoer naar het ziekenhuis (bv. de kosten voor uw auto, taxi, treinticket, enz.) zullen u vergoed worden met waardebonnen (1 waardebon/uitgevoerd onderzoeksbezoek na uw ontslag uit het ziekenhuis). Neem contact op met het studieteam voor praktische informatie.
- De sponsor zal het ziekenhuis/de onderzoeker vergoeden voor alle kosten voor bezoeken/consultaties, onderzoeken of behandelingen die specifiek zijn voor deze studie.
- U mag de onderzoeker contacteren of een lid van zijn/haar team, telkens wanneer u bijkomende informatie wenst.

Doelstellingen en beschrijving van het studieprotocol

Wij nodigen u uit om deel te nemen aan een klinische studie waarbij gebruik wordt gemaakt van menselijke levercellen (progenitorcellen genoemd) afkomstig van gedoneerde levers. Van deze cellen verwacht men dat ze een gecombineerd systemisch (in heel uw lichaam) en lokaal effect op de lever hebben. Men verwacht dat ze een immunomodulerende functie hebben, wat betekent dat ze de overdreven, ontstekingsreactie die bij uw ziekte werd vastgesteld helpen regelen en vooral een oplossing bieden voor het huidige acute event. De cellen die worden getest worden heterologe (van een andere persoon) progenitorcellen genoemd, afkomstig van de lever van een volwassen persoon (HHALPC) of HepaStem.

De cellen zijn stamcellen, die uit volwassen levers zijn geïsoleerd en ze zijn geen embryonale stamcellen. Ze worden op een specifieke wijze bewaard en zijn goedgekeurd door de gezondheidsinstanties om te worden gebruikt bij patiënten in het kader van een klinisch onderzoek.

Twaalf (12) patiënten zullen in Europa in deze studie worden opgenomen, waaronder ongeveer 6 in België. De onderzoeker zal aan patiënten voorstellen om deel te nemen, waarbij zoals bij u, een diagnose van levercirrose en acuut op chronisch leverfalen werd vastgesteld.

Het voornaamste doel van deze studie bestaat erin om de veiligheid en effectiviteit te beoordelen van twee (2) doseringen van HepaStem 4 maal toegediend in een periode van 14 dagen (om de 3 dagen 1 keer toegediend).

Deze studie is een open studie van 2 doseringsregimes. Dit betekent dat u ofwel
- 4 x een lage dosis cellen (250 miljoen cellen elk) in een periode van 14 dagen krijgt toegediend (dit betekent de toediening van 1 spuit van 50 ml tweemaal per week gedurende 2 weken) of

- 4 x een hoge dosis cellen (500 miljoen cellen in een periode van 14 dagen) (dit betekent de toediening van 2 spuiten van 50 ml tweemaal per week gedurende 2 weken)

In beide gevallen zullen u en uw onderzoeker op de hoogte zijn van de dosis die u zal worden toegediend.

Verloop van de studie

Na een behandeling van 2 weken met cellen en nog eens 2 weken tijdens de observatieperiode, zal u in de studie blijven tot 1 jaar voorbij is (follow-up periode). Binnen de eerste maand zal u 8 studiebeoordelingen krijgen, d.w.z. dagen met procedures en onderzoeken die specifiek aan de studie gekoppeld zijn. Tijdens de follow-up periode zal u 4 studiebeoordelingen krijgen.

De onderzoeken en procedures die in verband met de studie nodig zijn, worden hieronder beschreven. Enkele onderzoeken en procedures maken deel uit van de standaardzorg die in het ziekenhuis wordt verleend, terwijl anderen in het kader van de studie worden aangeboden.

Uw deelname aan de studie zal over 3 periodes verlopen:

1 Screeningsperiode: in die periode zal uw onderzoeker verifiëren of u in aanmerking komt om aan de studie deel te nemen en hij zal uw medische geschiedenis en de gegevens vergaren van enkele onderzoeken die werden gedaan, nadat u had toegestemd om aan de studie deel te nemen.

Er zullen ten hoogste 25 mL bloed (ongeveer 2 eetlepels bloed) worden afgenomen. Bloedstalen zijn reeds genomen in het kader van de zorgverlening in uw huidige klinische situatie; daarom zal geen bijkomende prik in uw arm worden gegeven om deze buisjes bloed te verzamelen.

Er zal ook een analyse van uw urine plaatsvinden.
De bloed- en urinestalen zullen ons toelaten om de parameters te analyseren die ons meer zullen zeggen over de staat van uw organen (lever, nieren, bloed), over uw virologische status (waaronder hepatitis en HIV) alsook uw immuniteit.
Indien het nog niet is gebeurd, zal een echografie worden genomen van uw lever en hart, X-stralen van uw borstkas en een electrocardiogram. Deze onderzoeken zijn niet-invasieve behandelingen en doen geen pijn.
Indien u tijdens uw opname in het ziekenhuis een leverbiopsie hebt gehad, zullen de resultaten van dit onderzoek worden verzameld.
U zal een “noodkaart” met informatie over deze studie ontvangen. Houd deze kaart steeds bij u. Indien u in het ziekenhuis wordt opgenomen, toon dan die kaart aan uw arts en informeer hem/haar dat u aan deze studie deelneemt.

Na de screeningsperiode, zal u, nadat aan alle criteria is voldaan om aan deze studie deel te nemen, in staat zijn om de actieve periode in de studie aan te vangen en HepaStem te krijgen.

2 Actieve periode: Tijdens deze periode zal u in de loop van 14 dagen 4 x HepaStem toegediend krijgen. Afhankelijk van de groep waartoe u behoort, zal u 250 of 500 miljoen cellen worden toegediend. Dit beantwoordt aan een volume van 50 of 100 mL vloeistof.

HepaStem zal intraveneus worden toegediend, wat betekent dat een naald in een kleine of grote ader zal worden geplaatst om het product toe te dienen. Voorafgaand aan de HepaStem infusie zal medicatie worden gegeven om slechte reacties van het lichaam op het product te voorkomen.

Tijdens het toedienen en in elk geval 2, 3 en 4 weken na de eerste toediening, zal u klinische onderzoeken ondergaan en zullen er bloedstalen worden genomen (max 15mL bloed, ongeveer 1 eetlepels bloed).
De bloedstalen zullen toelaten om informatie in te winnen over de status van uw organen (lever, nieren, bloed).
Er zal een echografie van uw hart worden gemaakt na de eerste toediening en een echografie van uw lever voor elke infusie, 2 en 4 weken na de eerste toediening.

3_ Follow-up periode: tijdens deze periode, zullen we u vragen om 2 maanden, 3 maanden, 6 maanden en 12 maanden na de eerste celinfusie naar het ziekenhuis terug te komen. Bij deze bezoeken zal u een klinisch onderzoek ondergaan en een bloedstaal (max 15mL bloed, ongeveer 1 eetlepels bloed). De resultaten van deze onderzoeken zullen worden verzameld.
De bloedstalen zullen ons toelaten om de parameters te analyseren die ons informatie zullen schenken over de status van uw organen (lever, nieren, bloed).
Indien u tijdens die periode een levertransplantatie hebt, zal indien mogelijk, een staal van uw verwijderde lever worden verzameld.

Na de follow-up periode, zullen we u vragen of u wilt instemmen om deel uit te maken van ons registratiesysteem voor bijkomende en regelmatige follow-up.

Risico's en ongemakken

A: Bijwerkingen van het medicijn van deze studie

- Alle medicijnen hebben gekende of onvoorspelbare bijwerkingen. Ook wanneer een voorafgaande studie heeft aangetoond dat HepaStem goed werd verdragen, kunt u toch de volgende bijwerkingen ervaren: **op korte termijn:**
  - trombose
- Ademhalingsmoeilijkheden overgevoeligheidsreactie of een reactie op de infusie: dit treedt op wanneer het immuunsysteem van het lichaam overmatig reageert op iets als medicatie. Overgevoeligheidsreacties kunnen omvatten: huidirritatie, roodheid, jeuk, zwelling, vochtafscheiding, korstvorming, huiduitslag, erupties, hoesten of kortademigheid, schorre stem, hoofdpijn, verstopte of lopende neus, niezen, rode (bloeddoorlopen) ogen, maagpijn, misselijkheid, braken, diarree, vermoeidheid, keelpijn, duizeligheid. Deze reacties kunnen schadelijk, ongemakkelijk of in sommige gevallen dodelijk zijn (in geval van anafylaxie): **op middellange of lange termijn:**
  - verspreiding in verschillende organen waar cellen de ontwikkeling van tumoren in de hand kunnen werken, hoewel dergelijke events zelden i.v.m. celtherapie werden gerapporteerd, een immuniteitsreactie aangezien HepaStem bestaat uit cellen van een andere persoon, wat eventueel tot afstoting van cellen kan leiden.

Andere risico's en ongemakken die voor het ogenblik niet bekend zijn, zouden zich kunnen voordoen. Het is daarom heel belangrijk dat elk nieuw gezondheidsprobleem vlug aan de onderzoeker wordt gerapporteerd, ongeacht of u al dan niet denkt dat het met de studie te maken heeft.

C: Risico's in verband met procedures die specifiek zijn voor deze studie

Er zijn ook risico's/ ongemakken, die verband houden met specifieke onderzoeken die in verband met deze studie zullen worden uitgevoerd:

- De intraveneuze toediening kan het volgende veroorzaken:

  - Pijn, zoals voor elke injectie.
  - Infectie. Elke opening in de huid houdt een risico van infectie in, hoewel intraveneuze injectie een aseptische procedure is.
  - Flebitis: ontsteking van een ader, die door infectie kan worden veroorzaakt.
  - Infiltratie: infiltratie doet zich voor wanneer een intraveneuze vloeistof of medicatie per toeval in het omgevende weefsel eerder dan in de ader terechtkomt.
  - Embolie: een bloedklonter of vaste massa, zoals ook een luchtbel, kan door een IV in de bloedsomloop terechtkomen en een bloedvat blokkeren. Evenwel, het is bijna onmogelijk om op een gevaarlijke manier lucht door een perifere IV te injecteren. Het risico is groter met een centrale IV.
- Het **afnemen van bloed,** noodzakelijk, voor een analyse kan (zelden) pijn veroorzaken, bloeden, blauwe plekken of infectie bij de plaats van de injectie tot gevolg hebben. Ook kunnen patiënten zich tijdens de procedure duizelig voelen of ze kunnen flauw vallen.

Het personeel dat bloed afneemt, zal de beste medische zorgen toedienen om ongemakken te voorkomen of ze tot een minimum te herleiden.

Bekendmaking van nieuwe informatie

Het kan gebeuren dat belangrijke nieuwe informatie bij het onderzoek m.b.t. HepaStem ter beschikking komt te staan. U zal op tijd worden geïnformeerd over elk nieuw element dat uw beslissing kan beïnvloeden om verder aan deze studie deel te nemen.

Indien u, in het licht van de nieuwe informatie, beslist om verder aan de studie deel te nemen, zal uw onderzoeker ervoor zorgen dat u verder de beste behandeling krijgt.

Contraceptie, zwangerschap en borstvoeding

Vrouwelijke deelnemer**:** omdat de gevolgen van HepaStem op een ongeboren kind of zuigeling niet echt gekend zijn, zal u niet worden toegelaten om aan deze klinische studie deel te nemen, wanneer u zwanger bent, wenst zwanger te worden of indien u borstvoeding geeft.

Indien u aan deze studie wenst deel te nemen, moet u één van de goedgekeurde contraceptiemethodes gebruiken (zodat u niet zwanger wordt). Uw arts zal de verschillende geschikte opties met u bespreken.

Baten

Indien u akkoord gaat om aan deze studie deel te nemen, kan HepaStem al dan niet blijken een gunstige werking te hebben bij de behandeling van uw ziekte of bij het verlichten van de symptomen, vooral bij het helpen oplossen van de huidige acute decompensatie van de leverfunctie.

De informatie die men dankzij deze studie krijgt, kan ertoe bijdragen om een betere kennis te krijgen van het gebruik van dit medisch product of van de ontwikkeling van een nieuw medisch product voor de behandeling van acuut-op chronisch leverfalen bij toekomstige patiënten.

Alternatieve behandeling:

Geen enkele door de overheden goedgekeurde behandeling van uw toestand, is voor het ogenblik in Europa beschikbaar.

De enige behandeling die nu beschikbaar is, is levertransplantatie. Evenwel, deze behandeling is ver van ideaal, wegens gebrek aan transplantatieorganen.

Terugtrekking uit de studie

Uw deelname is vrijwillig en u hebt het recht om u te allen tijde uit de studie terug te trekken, zonder dat u een reden opgeeft. Evenwel, het kan voor de onderzoeker en de sponsor van de studie nuttig zijn om te weten of u zich terugtrekt wegens de te grote last veroorzaakt door de behandeling (te veel onaangename bijwerkingen, bijvoorbeeld).

Het is ook mogelijk dat de onderzoeker u uit de studie haalt, omdat u zwanger bent, omdat hij/zij denkt dat het beter is voor uw gezondheid of omdat hij/zij er achter is gekomen dat u de instructies die aan de deelnemers werden gegeven niet opvolgt.

Tenslotte kunnen de bevoegde nationale of internationale overheden, het ethisch comité dat de studie eerst goedkeurde of de sponsor beslissen om de studie stop te zetten.

Als u uw toestemming om deel te nemen aan de studie intrekt, dan zullen, om de geldigheid van het onderzoek te garanderen, de gecodeerde gegevens tot het punt waarop u zich terugtrok, bewaard blijven. Er mogen geen nieuwe gegevens naar de sponsor verzonden worden.

Als u uw goedkeuring om deel te nemen aan de studie intrekt, kunt u contact opnemen met de onderzoeker en vragen om de stalen die nog niet gebruikt zijn, te laten vernietigen. De resultaten verkregen uit uw stalen voordat u uw goedkeuring introk, blijven eigendom van de studiesponsor.

Behandeling nadat de studie werd stopgezet

In al deze situaties van terugtrekking uit de studie, maar ook wanneer de geplande periode van uw deelname is beëindigd, zal uw onderzoeker de staat van uw gezondheid onderzoeken en de best beschikbare behandeling voorschrijven.

Stalen van biologisch materiaal dat gedurende de studie werd verzameld

De sponsor van deze studie verzekert dat de stalen (zoals bloed, urine, weefsel van de lever) alleen in het kader van de studie zullen worden gebruikt.

- De procedure voor het coderen van stalen is dezelfde als deze die wordt gebruikt voor uw medische gegevens. Stalen die naar de sponsor worden verstuurd, zullen daarom alleen uw studie-ID-code bevatten.
- De beheerder van deze stalen (het hematologielaboratorium van de Cliniques St. Luc in Brussel en het Translational Research Center, KU Leuven, Leuven) zal ze gebruiken binnen de context van klinisch onderzoek en ze vernietigen aan het eind van de geplande bewaarperiode.
- Het staal van afgenomen biologisch materiaal wordt beschouwd als een ‘donatie’ en u moet er zich van bewust zijn dat u, in principe, geen enkel financieel voordeel (royalty’s) zult ontvangen geassocieerd met de ontwikkeling van nieuwe therapieën die zijn afgeleid van het gebruik van uw donatie van biologisch materiaal en die mogelijk commerciële waarde kunnen hebben.

Het overschot van stalen zal worden vernietigd zodra de analyses die in dit document zijn beschreven, werden uitgevoerd (ten laatste, één jaar na beëindiging van de studie)

Vertrouwelijkheid en gegevensbescherming

Uw deelname aan de studie betekent dat u ermee akkoord gaat dat de onderzoeker gegevens over u verzamelt en dat de studiesponsor deze gegevens gebruikt voor onderzoeksdoeleinden en in verband met wetenschappelijke en medische publicaties.

U hebt het recht om de onderzoeker te vragen welke gegevens er over u verzameld worden en waarvoor ze in functie van de studie gebruikt worden. Deze gegevens omvatten uw huidige klinische situatie, maar ook wat achtergrondgegevens, de resultaten van onderzoeken uitgevoerd binnen de

context van uw gezondheidszorg in overeenstemming met de huidige standaarden en natuurlijk ook de resultaten van onderzoeken vereist door het protocol. U hebt het recht om deze gegevens te inspecteren en verbeteren als ze fout zouden zijn. Deze rechten zijn gegarandeerd door de wet van 8 december 1992 op de bescherming van de persoonlijke levenssfeer in functie van het verwerken van persoonlijke gegevens en door de wet van 22 augustus 2002 over de rechten van de patiënt.

De onderzoeker is gebonden door het beroepsgeheim over wat de verzamelde gegevens betreft.

Dit betekent dat hij/zij verplicht is om nooit uw naam te vermelden in de context van een publicatie of conferentie en dat hij/zij ook uw gegevens zal coderen (uw identiteit zal vervangen worden door een ID-code in de studie) alvorens ze naar de beheerder van de database van verzamelde gegevens te versturen (Clinical Department, Promethera Biosciences).

De onderzoeker en zijn/haar team zullen daarom de enigen zijn die een link kunnen maken tussen de gegevens die tijdens de studie verzonden worden en uw medisch dossier. Voor deze studie vereist de wet dat deze link met uw dossier gedurende minstens 30 jaar en maximaal 50 jaar bewaard blijft, in overeenstemming met de Belgische wet van 19 december 2008 over het gebruik van menselijk biologisch materiaal en de van toepassing zijnde koninklijke besluiten. De persoonlijke gegevens die verstuurd worden, zullen geen combinatie van elementen bevatten waaruit u geïdentificeerd kunt worden.

Voor de beheerder van de studiegegevens die wordt aangeduid door de sponsor, zullen de verstuurde gegevens het niet mogelijk maken om u te identificeren. De beheerder is verantwoordelijk voor het verzamelen van de gegevens van alle onderzoekers die deelnemen aan deze studie en ze te verwerken en beschermen in overeenstemming met de vereisten van de Belgische wet op de bescherming van de privacy.

Om de kwaliteit van de studie te verifiëren, is het mogelijk dat uw medisch dossier onderzocht wordt door personen die gebonden zijn door het beroepsgeheim en aangewezen door het ethisch comité, de sponsor van de studie of een onafhankelijk controle-orgaan. In elk geval mag dit onderzoek van uw medisch dossier alleen plaatsvinden onder de verantwoordelijkheid van de onderzoeker en onder toezicht van één van de door hem/haar aangeduide medewerkers.

De (gecodeerde) studiegegevens zullen mogelijk verstuurd worden naar Belgische of andere regelgevende autoriteiten, de relevante ethische comités, andere artsen en/of organisaties die samenwerken met de sponsor.

Ze zullen mogelijk ook verstuurd worden naar andere vestigingen van de sponsor in België en in andere landen waar de standaarden voor het beschermen van persoonlijke gegevens mogelijk anders of minder streng zijn. Zoals hierboven uitgelegd, worden de verstuurde gegevens gecodeerd. De sponsor verbindt zich ertoe de beperkingen van de Europese Richtlijn en de Belgische wetgeving op de bescherming van de privacy te respecteren.

Uw toestemming om deel te nemen aan deze studie impliceert hierdoor uw toestemming voor het gebruik van uw gecodeerde medische gegevens voor het doel beschreven in dit informatieformulier en voor de overdracht ervan naar de hierboven vermelde personen en autoriteiten.

De sponsor verbindt zich ertoe om de gegevens alleen te gebruiken binnen de context van de studie waaraan u deelneemt.

Verzekering

Elke deelname aan een klinische studie houdt een risico in, hoe klein ook. Zelfs zonder fout aanvaardt de sponsor verantwoordelijkheid voor schade die aan de deelnemer wordt veroorzaakt (of in het geval van overlijden, zijn/haar rechthebbenden) en die rechtstreeks of onrechtstreeks

verbonden is met zijn/haar deelname aan de studie. De sponsor heeft een verzekering afgesloten voor deze verantwoordelijkheid.

We vragen u daarom om elk nieuwe gezondheidsprobleem te melden aan de onderzoeker voordat u een andere arts raadpleegt, andere medicatie begint in te nemen of een andere medische behandeling ondergaat. Als u, om eender welke reden, een andere arts raadpleegt tijdens deze klinische studie, moet u hem/haar inlichten dat u deelneemt aan een klinische studie/uw deelnemerskaart van de klinische studie tonen. Dit kan belangrijk zijn voor het stellen van een diagnose en het behandelen van uw klachten.

Als de onderzoeker denkt dat een verband met de studie mogelijk is (de verzekering dekt het natuurlijke verloop van uw ziekte of de bekende bijwerkingen van uw normale behandeling niet), zal hij/zij de studiesponsor informeren, die de aangifteprocedure bij de verzekeringsmaatschappij zal opstarten. De laatste zal een deskundige aanstellen (als dit nodig wordt geacht) om te beoordelen of er een verband bestaat tussen uw nieuwe gezondheidsproblemen en de studie.

In geval van onenigheid met de onderzoeker of met de door de verzekeringsmaatschappij aangestelde deskundige en ook wanneer u dat nodig acht, kunt u, of kunnen uw nabestaanden in geval van overlijden, de verzekeraar rechtstreeks in België dagvaarden (naam van verzekeringsmaatschappij, polisnummer, contactpersoon).

De wet voorziet dat de verzekeraar voor de rechtbank moet verschijnen van de locatie waar het schadegeval optrad, voor de rechtbank van uw woonplaats, of voor de rechtbank van de hoofdzetel van de verzekeraar.

Indien u aan deze klinische studie deelneemt, verzoeken wij om:

- Ten minste 5 weken voor het begin van uw deelname geen alcohol te drinken.
- Volledig mee te werken aan een rimpelloos verloop van de studie.
- Geen enkele informatie in verband met uw gezondheidstoestand, de medicatie die u neemt of de symptomen die u ondervindt, te verbergen.
- Niet deel te nemen aan een andere klinische studie waarbij er sprake is van een behandeling in het kader van het onderzoek, of het nu gaat om een medisch hulpmiddel of een procedure, terwijl men aan deze studie deelneemt.
- De "noodkaart" steeds bij u te hebben. Dit is absoluut noodzakelijk voor uw veiligheid in geval u dringende zorg nodig hebt in een instelling die u niet kent.

Contact

Indien u verder informatie nodig hebt, maar ook indien u problemen of zorgen heeft, kunt u de onderzoeker ……of een lid van zijn/haar research team..... op het volgende telefoonnummer (xx / xxx-xx-yy).....contacteren.

In noodgeval of buiten de consultatie-uren, contacteer de ombudsman …… op het volgend telefoonnummer ……... en vermeld dat u aan een klinische studie deelneemt. Uw dossier zal informatie bevatten die nuttig is voor de dienstdoende arts in verband met deze klinische studie.

HEP101 STUDIE

MULTICENTRALE FASE II VEILIGHEIDSSTUDIE EN EFFECTIVITEIT-VOORSTUDIE VAN 2 DOSERINGSREGIMES VAN HEPASTEM BIJ PATIËNTEN MET ACUUT-OP-CHRONISCH LEVERFALEN

Toestemming van de geïnformeerde patiënt

ID van de patiënt in de studie: ……..

Deelnemer

Ik verklaar dat ik ingelicht ben over de aard van de studie, haar doelstelling, duur, risico's en baten, alsook over wat van mij wordt verwacht. Ik heb kennis genomen van het informatiedocument en ik heb voldoende tijd gehad om erover na te denken en te bespreken met een persoon van mijn keuze, zoals mijn huisarts of een familielid.

Ik heb de gelegenheid gehad om aan de onderzoeker de vragen te stellen die bij me opkwamen en ik heb een bevredigend antwoord op mijn vragen gekregen.

Ik begrijp dat mijn deelname aan deze studie vrijwillig is en dat ik vrij ben om mijn deelname aan deze studie te beëindigen zonder dat mijn relatie met het therapeutisch team dat verantwoordelijk is voor mijn gezondheid, in het gedrang komt.

Ik ben er me van bewust dat voor deze klinische studie mijn persoonlijke gegevens, vooral mijn medische gegevens worden verzameld, opgeslagen en geanalyseerd. Het gebruik van de informatie in verband met mijn gezondheid stemt overeen met de wettelijke bepalingen en vereist, voorafgaand aan deze studie een vrijwillig gegeven geïnformeerde toestemming. Zonder daaropvolgende toestemming kan ik niet aan de studie deelnemen.

De persoonlijke gegevens zijn geëncrypteerd. Ik heb het recht mijn gegevens te bekijken/te verbeteren.

De toestemming om mijn persoonlijke gegevens te verzamelen en te verwerken, vooral informatie over mijn gezondheid, is onherroeppelijk. Ik ben reeds geïnformeerd dat ik mijn deelname aan dit onderzoek te allen tijde kan beëindigen. Ingeval van een dergelijke terugtrekking van mijn toestemming, stem ik ermee in dat mijn gegevens die tot hiertoe zijn verzameld, onder pseudoniem (geëncrypteerd) kunnen worden geanalyseerd.

Ik stem ermee in dat mijn gegevens na beëindiging van de studie of bij onderbreking zullen worden opgeslagen volgens de nationale vereisten.

Ik stem ermee in dat de gegevens van deze studie, verzameld voor de doelstellingen van deze studie, op een later datum worden verwerkt, op voorwaarde dat de verwerking uitsluitend verband houdt met het kader van de huidige studie voor een beter begrip van de ziekte en haar behandeling.

Ik stem ermee in dat de sponsor stalen behoudt van het biologisch materiaal dat tijdens de studie, op het einde van het klinisch onderzoek voor verdere onderzoeksdoeleinden werd verzameld, maar binnen het kader van de huidige studie.

Ik stem ermee in dat mijn huisarts of andere specialisten die verantwoordelijk zijn voor mijn gezondheid, worden geïnformeerd over mijn deelname aan deze klinische studie.

Ik heb een kopie ontvangen van de informatie voor de deelnemer en een geïnformeerd toestemmingsformulier.

Achternaam van de patiënt: ……Voornaam: ……

Handtekening: …… Datum: ……

Getuige/Tolk

Ik was aanwezig tijdens het volledige proces van de patiënteninformatie en ik bevestig dat de informatie over de doelstellingen en de procedures van de studie naar behoren werd verleend, dat de deelnemer (of zijn/haar vertegenwoordiger) blijkbaar de studie begreep en dat de toestemming om deel te nemen in volle vrijheid werd gegeven.

De getuige/tolk: … Achternaam & Voornaam: ……..

Handtekening: …… Datum : ……

De onderzoeker

Ik, de ondergetekende,……., onderzoeker, bevestig dat ik reeds mondeling de nodige informatie over de studie heb verstrekt en dat ik aan de deelnemer een kopie van het informatief document heb gegeven.

Ik bevestig dat er geen druk werd uitgeoefend om de patiënt te overtuigen om zijn toestemming te geven om aan deze studie deel te nemen en dat ik, zo nodig, bijkomende vragen wil beantwoorden.

Ik bevestig dat ik in overeenstemming met ethische principes handel die zijn opgenomen in de laatste versie van de “Verklaring van Helsinki”, de “Goede Klinische Praktijk” en de huidige nationale regeling, in verband met onderzoeken waaraan mensen deelnemen.

Achternaam van de onderzoeker: ……Voornaam……

Handtekening: …… Datum: ……

ÉTUDE HEP101

ÉTUDE CLINIQUE MULTICENTRIQUE DE PHASE II VISANT A EVALUER LA SECURITE ET L'EFFICACITE PRELIMINAIRE DE 2 DOSES D'HEPASTEM CHEZ DES PATIENTS SOUFFRANT D'INSUFFISANCE HEPATIQUE AIGUË SUR UNE MALADIE CHRONIQUE DU FOIE.

Formulaire d'information pour le patient

Ce formulaire d'information pour le patient et le formulaire de consentement éclairé sont destinés aux patients souffrant d'une insuffisance hépatique aiguë sur maladie chronique du foie (ACLF) qui sont invités à participer à une étude de recherche clinique réalisée avec HepaStem

Promoteur de l'étude : **Promethera Biosciences**
Adresse : **Rue Granbonpré 11**
1435 Mont St Guibert
Belgique

Investigateur principal : _____
Adresse : _____
Numéro de téléphone : _____

Introduction

Vous êtes invité(e) à participer à une étude clinique destinée à évaluer un médicament expérimental pour le traitement de votre maladie. Un médicament expérimental est un médicament faisant encore l'objet d'études pour évaluer son efficacité, sa sécurité d'emploi ou son mécanisme d'action.

L'investigateur (ou médecin de l'étude, c.-à-d. le médecin responsable de cette étude de recherche à l'hôpital) et le promoteur (la firme qui initie et finance cette recherche) espèrent que ce médicament expérimental peut présenter des avantages pour le traitement de patients atteints de la même maladie que la vôtre. Néanmoins, rien ne garantit que vous tiriez un bénéfice de votre participation à cette étude.

Avant que vous n'acceptiez de participer à cette étude, nous vous invitons à prendre connaissance de ses implications en termes d'organisation, risques et bénéfices éventuels, afin que vous puissiez prendre une décision en toute connaissance de cause. C'est ce qu'on appelle donner un « consentement éclairé ».

Veuillez lire attentivement ces quelques pages d'information et poser toutes les questions que vous souhaitez à l'investigateur ou à la personne qui le représente. Ce document comprend 2 parties : l'information essentielle à votre prise de décision et votre formulaire de consentement écrit.

Avant de prendre une décision, vous devez savoir que :

- Cette étude clinique est mise en œuvre après avoir fait l'objet d'une évaluation par un comité d'éthique (de Louvain) après consultation des comités d'éthique d'autres centres participants.
- Votre participation est volontaire et doit rester libre de toute contrainte. Elle nécessite la signature d'un document exprimant votre consentement. Même après avoir signé ce document, vous pouvez interrompre votre participation en informant le médecin investigateur. Votre décision de ne pas ou de ne plus participer à l'étude n'aura aucun impact sur la qualité de vos soins ni sur vos relations avec le médecin investigateur.

- Les données recueillies au cours de l'étude sont confidentielles. Votre anonymat sera toujours garanti, y compris lors de la publication des résultats.
- Le promoteur a souscrit une assurance au cas où vous subiriez un dommage lié à votre participation à cette étude clinique.

  Compagnie d'assurances : QBE Syndicate
  Numéro de la police : 16ME305419EA
- Votre participation à cette étude est totalement gratuite. Aucun frais ne vous sera facturé pour les examens spécifiques liés à cette étude. Cependant, vous ne serez pas rémunéré(e) pour votre participation à cette étude.
- Les dépenses liées à votre transport jusqu'à l'hôpital (comme les frais de voiture, de taxi, le ticket de train, etc.) vous seront remboursées avec des vouchers (1 pièce/visite de l'étude effectuée après votre sortie de l'hôpital). Veuillez contacter le personnel de l'étude pour les détails pratiques.
- Le promoteur remboursera l'hôpital/médecin investigateur pour tous les frais de visites/consultations, examens et traitements spécifiques à l'étude.
- Vous pouvez contacter le médecin investigateur ou un membre de son équipe à tout moment si vous avez besoin d'informations complémentaires.

Objectifs et description du protocole de l'étude

Vous êtes invité(e) à participer à une étude clinique portant sur le traitement par des cellules de foie humain (appelées « cellules progénitrices ») provenant de foies de donneurs. On espère que ces cellules ont un effet combiné systémique (dans tout le corps) et local dans le foie. Nous pensons qu'elles jouent un rôle immunomodulateur, c.-à-d. qu'elles aident à réguler la réponse inflammatoire exagérée qui est observée au cours de votre maladie, en particulier qu'elles aident à résoudre l'épisode aigu actuel de votre affection. Les cellules testées sont appelées « cellules progénitrices allogéniques (c.-à-d. provenant d'une autre personne) isolées à partir de foie humain adulte » ou HepaStem.
Les cellules sont des cellules souches isolées à partir de foies adultes et ne sont pas des cellules souches embryonnaires. Elles sont conservées selon une méthode spécifique et les autorités de santé ont approuvé leur utilisation chez des patients dans le cadre d'une étude clinique.

Douze (12) patients seront inclus dans cette étude en Europe, dont environ 6 en Belgique. Le médecin investigateur proposera à des patients qui, comme vous, présentent un diagnostic de cirrhose et une insuffisance hépatique aiguë sur maladie chronique du foie, de participer à cette étude.

L'objectif principal de cette étude est d'évaluer la sécurité et l'efficacité de deux (2) posologies d'HepaStem administrées 4 fois sur une période de 14 jours (1 administration tous les 3 jours).

Il s'agit d'une étude réalisée en ouvert pour évaluer 2 schémas posologiques, ce qui signifie que vous recevrez

- 4 administrations de la dose faible de cellules (250 millions de cellules pour chaque administration) sur une période de 14 jours (cela signifie l'administration d'1 seringue de 50 ml deux fois par semaine pendant 2 semaines), ou
- 4 administrations de la dose élevée de cellules (500 millions de cellules pour chaque administration) sur une période de 14 jours (cela signifie l'administration de 2 seringues de 50 ml deux fois par semaine pendant 2 semaines).

Dans les deux cas, vous et votre médecin investigateur saurez quelle posologie vous recevez.

Déroulement de l'étude

Après la phase de traitement de 2 semaines par les cellules et une période supplémentaire de 2 semaines de surveillance, vous resterez encore dans l'étude pendant 1 an (phase de suivi). Au cours du premier mois, vous ferez l'objet de 8 évaluations d'étude, c.-à-d. que vous subirez ces jours-là des procédures et des examens spécifiques à l'étude. Pendant la phase de suivi, vous ferez l'objet de 4 évaluations d'étude.

Les examens et procédures nécessaires liés à l'étude sont décrits ci-dessous. Certains de ces examens et procédures font partie des soins standards prodigués par votre hôpital, tandis que d'autres sont offerts dans le cadre de cette étude.

Votre participation à l'étude sera constituée de 3 phases :

1 Phase de sélection : pendant cette phase, votre médecin investigateur vérifiera si vous êtes éligible pour participer à cette étude, et établira un relevé de vos antécédents médicaux et des résultats de certains examens réalisés après avoir reçu votre accord pour participer à l'étude.

Tout au plus 25 ml supplémentaires de sang (environ 2 cuillères à soupe) seront prélevés. Des prises de sang sont déjà réalisées dans le cadre des soins liés à votre situation clinique actuelle ; aucune piqûre supplémentaire ne sera donc réalisée sur votre bras pour prélever ces tubes de sang.
Certaines analyses d'urine seront également réalisées.
Les échantillons de sang et d'urine permettront d'analyser des paramètres qui fourniront des informations sur l'état de vos organes (foie, reins, sang), sur votre statut virologique (notamment pour l'hépatite et l'infection à VIH) et sur votre immunité.
Si ce n'est pas déjà fait, on réalisera une échographie de votre foie et de votre cœur, une radiographie du thorax et un électrocardiogramme. Ces examens ne sont pas invasifs et ils sont indolores.
Si vous avez déjà subi une biopsie du foie pendant cette hospitalisation, les résultats de cet examen seront collectés.
Vous recevrez une « carte d'urgence » mentionnant toutes les informations sur l'étude. Veuillez porter cette carte sur vous en permanence. Si vous n'êtes pas hospitalisé(e), veuillez montrer cette carte à votre médecin et l'informer que vous participez à cette étude.

Après la phase de sélection, si vous répondez à tous les critères nécessaires pour entrer dans l'étude, vous serez en mesure de débuter la phase active de l'étude et vous recevrez le médicament HepaStem.

2 Phase active de l'étude : Pendant cette phase, vous recevrez 4 administrations d'HepaStem sur une période de 14 jours. En fonction du groupe auquel vous appartenez, vous recevrez 250 ou 500 millions de cellules par administration, ce qui équivaut à un volume de 50 ou 100 ml de liquide.

HepaStem sera administré par voie intraveineuse, ce qui signifie qu'une aiguille sera introduite dans une veine de petit ou grand calibre pour administrer le médicament. En vue de prévenir les réactions indésirables de votre corps au médicament, un médicament sera administré avant la perfusion d'HepaStem.

Pendant chaque administration et dans tous les cas, 2, 3 et 4 semaines après la 1ère administration, vous ferez l'objet d'examens cliniques et des échantillons de sang seront

prélevés (environ 15 mL de sang soit 1 cuillère à soupe). Les échantillons de sang permettront d'obtenir des informations sur l'état de vos organes (foie, reins, sang).
Une échographie de votre cœur sera réalisée après la 1ère administration et une échographie de votre foie sera réalisée après avant chaque perfusion, puis 2 et 4 semaines après la 1ère administration.

3_ Phase de suivi : pendant cette période, vous serez invité(e) à revenir à l'hôpital 2 mois, 3 mois, 6 mois et 12 mois après l'administration de la 1ère perfusion de cellules. Au cours de ces visites, vous ferez l'objet d'un examen clinique et un échantillon de sang sera prélevé (environ 15 mL de sang soit 1 cuillère à soupe). Les résultats de ces examens seront collectés.
Les échantillons de sang permettront d'analyser des paramètres qui fourniront des informations sur l'état de vos organes (foie, reins, sang).
Pendant cette période, si vous avez subi une transplantation du foie, un échantillon de votre foie explanté sera collecté si possible.

Après la phase de suivi, nous vous demanderons si vous acceptez d'être inscrit(e) dans notre registre dans le cadre d'un suivi régulier et complémentaire.

Risques et inconvénients

A : Effets secondaires du médicament de l'étude

- Tous les médicaments ont des effets secondaires connus ou imprévisibles. Même si l'étude précédente a révélé qu'HepaStem était bien toléré, il est toujours possible que vous présentiez les effets secondaires suivants : **à court terme :**
  - Thrombose
- Affection respiratoire Réaction d'hypersensibilité ou réaction à la perfusion : ceci se produit lorsque le système immunitaire du corps sur-réagit à quelque chose comme un médicament. La réaction d'hypersensibilité peut comprendre : irritation de la peau, rougeur, démangeaisons, gonflement, suintement, formation de croûtes, éruptions, toux ou essoufflement, enrouement de la voix, maux de tête, nez bouché ou qui coule, éternuements, yeux rouges, douleurs à l'estomac, nausée, vomissements, diarrhée, fatigue, mal de gorge, vertiges. Ces réactions peuvent être préjudiciables, inconfortables ou occasionnellement, fatales (en cas d'anaphylaxie). **à moyen ou long terme :**
  - distribution dans différents organes où les cellules peuvent favoriser le développement d'une tumeur, même si ces effets ont été rarement rapportés avec la thérapie cellulaire réaction immunitaire car HepaStem est constitué de cellules provenant d'une autre personne, ce qui peut éventuellement induire une réaction de rejet des cellules.

D'autres risques et inconvénients inconnus à ce jour pourraient également apparaitre. Il est donc très important de signaler rapidement tout nouveau problème de santé au médecin investigateur, que vous pensiez ou non qu'il soit en rapport avec l'étude.

C : Risques associés aux procédures spécifiques à l'étude

Il existe également des risques/inconvénients associés aux examens spécifiques qui seront réalisés dans le cadre de cette étude :

- L'administration intraveineuse peut provoquer les effets suivants :
  - Douleur, comme pour toute injection.

- Infection. Toute ouverture au niveau de la peau peut comporter un risque d'infection, même si l'insertion intraveineuse est une procédure aseptique.
- Phlébite : inflammation d'une veine pouvant être causée par une infection.
- Infiltration : une infiltration survient lorsqu'un liquide ou un médicament administré par voie intraveineuse pénètre accidentellement dans le tissu environnant plutôt que dans la veine.
- Embolie : un caillot sanguin ou une autre particule solide, ou encore une bulle d'air, peut être introduit dans la circulation au cours de l'administration IV et bloquer finalement un vaisseau. Cependant, il est presque impossible d'injecter de l'air au cours d'une administration IV par voie périphérique. Le risque est plus élevé en cas d'administration IV par voie centrale.

- La **prise de sang** nécessaire aux analyses peut (rarement) causer une douleur, un saignement, une contusion (bleu) ou une infection autour du site d'injection. Chez certains patients, des étourdissements, ou même un évanouissement, peuvent survenir pendant la procédure.

L'équipe qui réalisera la prise de sang fera usage des meilleurs soins médicaux pour empêcher ou réduire au minimum ces inconforts.

Notification de nouvelles informations

Il est possible que, pendant le déroulement de l'étude clinique, de nouvelles informations importantes sur HepaStem, le médicament étudié, deviennent disponibles. Vous serez informé(e) au moment opportun de tout élément nouveau susceptible de modifier votre décision de poursuivre votre participation à cette étude.

Si, au vu de ces nouvelles informations, vous décidez d'interrompre votre participation à l'étude, votre médecin investigateur veillera à ce que vous continuiez à recevoir le meilleur traitement possible.

Contraception, grossesse et allaitement

Participant de sexe féminin : Étant donné que les effets d'HepaStem sur un enfant à naître ou un nourrisson ne sont pas parfaitement connus, vous ne serez pas autorisée à participer à cette étude clinique si vous êtes enceinte, si vous souhaitez tomber enceinte ou si vous allaitez.

Si vous choisissez de participer à cette étude, vous devrez utiliser l'une des méthodes contraceptives autorisées (de manière à ne pas tomber enceinte). Votre médecin discutera avec vous des différentes options adéquates.

Bénéfices

Si vous acceptez de participer à cette étude, HepaStem pourra ou non s'avérer bénéfique pour le traitement de la maladie dont vous êtes atteint(e) ou soulager vos symptômes, en particulier pour aider à résoudre la décompensation aiguë actuelle de la fonction de votre foie.

Les informations obtenues grâce à cette étude peuvent contribuer à une meilleure connaissance de l'utilisation de ce médicament ou au développement d'un nouveau médicament pour le traitement de l'insuffisance hépatique aiguë sur maladie chronique du foie chez de futurs patients.

Traitement alternatif :

À ce jour, aucun traitement de votre affection approuvé par les autorités n'est disponible en Europe.

Le seul traitement actuellement disponible est la greffe de foie. Cependant, ce traitement est loin d'être idéal vu le manque d'organes pour la transplantation.

Retrait de l'étude

Votre participation est volontaire et vous avez le droit de vous retirer de l'étude à tout moment et sans devoir vous justifier. Néanmoins, il peut être utile pour le médecin investigateur et pour le promoteur

de l'étude de savoir si vous vous retirez de l'étude car les contraintes liées au traitement sont trop importantes (par exemple, trop d'effets secondaires désagréables).

Il est aussi possible que le médecin investigateur vous retire de l'étude car vous êtes enceinte, car il/elle pense que c'est mieux pour votre santé ou car il/elle constate que vous ne respectez pas les consignes données aux participants.

Enfin, il est également possible que les autorités compétentes nationales ou internationales, le comité d'éthique qui a initialement approuvé l'étude ou le promoteur, décident d'interrompre l'étude.

Si vous retirez votre consentement à participer à l'étude, afin de garantir la validité de la recherche, les données encodées jusqu'au moment du retrait, seront conservées. Aucune nouvelle donnée ne sera envoyée au promoteur.

Si vous retirez votre consentement à participer à l'étude, vous pouvez contacter l'investigateur et demander que vos échantillons qui n'ont pas encore été utilisés, soient détruits. Les résultats obtenus de vos échantillons avant le retrait de votre consentement, restent la propriété du promoteur de l'étude.

Traitement après l'arrêt de l'étude

Dans toutes ces situations de retrait de l'étude mais également lorsque la période de participation prévue est arrivée à son terme, votre médecin investigateur évaluera votre état de santé et vous prescrira le meilleur traitement disponible.

Échantillons de matériel biologique collectés au cours de l'étude

Le promoteur de l'étude s'engage à ce que les échantillons (p. ex. sang, urine, tissu hépatique) ne soient utilisés que dans le cadre de l'étude.

- La procédure de codage des échantillons est la même que celle utilisée pour vos données médicales. Les échantillons envoyés au promoteur ne comprendront donc que votre code ID de l'étude.

- Le gestionnaire de ces échantillons (laboratoire d'hématologie des Cliniques St Luc, Bruxelles et Translational Research Center, KU Leuven, Louvain) s'engagent à les utiliser dans le contexte de la recherche clinique et de les détruire à la fin de la période de stockage prévue.

- L'échantillon de matière biologique prélevé est considéré comme un « don » et vous devez savoir que, en principe, vous ne recevrez aucun avantage financier (royalties) associé au développement d'un nouveau traitement dérivé de l'utilisation de votre don de matière biologique, qui pourrait présenter une valeur commerciale.

Le surplus de vos échantillons sera détruit dès que les analyses décrites dans ce document auront été réalisées (au plus tard, un an après la fin de l'étude).

Confidentialité et protection des données

Votre participation à l'étude signifie que vous consentez à la collecte de données à votre sujet par l'investigateur et à l'utilisation de ces données par le promoteur à des fins de recherches et en lien avec des publications scientifiques et médicales.
Vous avez le droit de demander à l'investigateur quelles données sont collectées à votre sujet et quelle est leur utilisation dans le cadre de l'étude. Ces données concernent votre état clinique actuel, mais également une partie de vos antécédents, les résultats des examens réalisés dans le contexte de votre prise en charge selon les standards actuels et évidemment, les résultats des examens requis par le protocole. Vous avez le droit de consulter ces données et de les corriger si elles sont incorrectes. Ces droits sont garantis par la loi du 8 décembre 1992 relative à la protection de la vie privée à l'égard du traitement de données à caractère personnel et par la loi du 22 août 2002 relative aux droits du patient.
L'investigateur a un devoir de confidentialité vis-à-vis des données collectées.

Cela signifie qu'il/elle s'engage, non seulement à ne jamais révéler votre nom dans le contexte d'une publication ou d'une conférence, mais également qu'il/elle codera (votre identité sera remplacée par un code ID dans l'étude) vos données avant de les envoyer au gestionnaire de la banque des données collectées (Clinical Department, Promethera Biosciences).
L'investigateur et son équipe seront donc les seules personnes capables d'établir un lien entre les données transmises au cours de l'étude et votre dossier médical. Pour la présente étude, la loi exige que ce lien avec votre dossier soit conservé pendant minimum 30 ans et maximum 50 ans, loi belge du 19 décembre 2008 relative à l'obtention de matériel biologique humain et les arrêtés royaux qui s'y rapportent.
Les données personnelles transmises ne contiendront aucune combinaison d'éléments qui pourraient permettre de vous identifier.
Pour le gestionnaire des données de l'étude désigné par le promoteur, les données transmises ne permettront pas de vous identifier. Ce dernier est responsable de la collecte des données recueillies par tous les investigateurs participant à l'étude, leur traitement et leur protection selon les exigences de la loi belge relative à la protection de la vie privée.
Afin de vérifier la qualité de l'étude, il est possible que vos dossiers médicaux soient examinés par des personnes soumises au secret professionnel et désignées par le comité d'éthique, le promoteur de l'étude ou un audit indépendant. Dans tous les cas, l'examen de votre dossier médical sera sous l'entière responsabilité de l'investigateur et sous la supervision de l'un de ses collaborateurs, qu'il/elle aura désigné.
Les données de l'étude (codées) pourront être envoyées à des autorités de règlementation belges ou autres, aux comités d'éthique pertinents, à d'autres médecins et/ou à des organisations travaillant en collaboration avec le promoteur.
Elles pourront également être envoyées vers d'autres sites du promoteur en Belgique et dans d'autres pays où les standards en termes de protection des données personnelles peuvent être différents ou moins rigoureux. Comme indiqué ci-dessus, les données transmises sont codées. Le promoteur s'engage donc au respect des contraintes de la directive européenne et de la législation belge sur la protection de la vie privée.
Votre consentement à participer à la présente étude implique par conséquent également, votre consentement à l'utilisation de vos données médicales codées pour les objets décrits dans le présent feuillet d'information et à leur transmission aux personnes et autorités mentionnées ci-dessus.
Le promoteur s'engage à n'utiliser les données collectées que dans le contexte de l'étude à laquelle vous participez.

Assurance

Toute participation à une étude clinique comprend des risques, aussi faibles soient-ils. Même si aucune erreur n'a été commise, le promoteur assume la responsabilité de tout dommage causé au participant (ou dans le cas d'un décès, ses ayants-droits) et lié directement ou indirectement à sa participation à l'étude. Le promoteur a souscrit une assurance couvrant cette responsabilité.
Il vous est donc demandé d'informer l'investigateur de tout nouveau problème de santé avant de consulter un autre médecin, de prendre un autre médicament ou de recevoir tout autre traitement médical. Si pour une raison quelconque, vous consultez un autre médecin pendant la présente étude clinique, vous devez l'informer que vous participez à une étude clinique/lui présenter votre carte de participant à une étude clinique. Ceci peut être important pour établir un diagnostic et traiter vos plaintes.
Si l'investigateur pense d'un lien avec l'étude est possible (l'assurance ne couvre pas la progression naturelle de votre maladie ou les effets secondaires connus de votre traitement normal), il/elle en informera le promoteur de l'étude, qui initiera la procédure de déclaration à la compagnie

d'assurance. Cette dernière nommera un expert – si elle le considère nécessaire – pour établir s'il existe un lien entre votre nouveau problème de santé et l'étude.
Dans le cas d'un désaccord avec l'investigateur ou l'expert désigné par la compagnie d'assurance, et également si vous en ressentez la nécessité, vous ou – en cas de décès - vos ayants-droits pouvez entamer une procédure à l'égard de l'assureur, directement en Belgique (nom de la compagnie d'assurance, numéro de la police, contact).
La loi assure que l'assureur peut être convoqué par le juge de l'endroit où l'événement donnant lieu au dommage s'est déroulé, ou devant le juge de votre domicile, ou devant le juge du siège social de l'assureur.

Si vous participez à cette étude clinique, nous vous demandons :

- De ne pas boire d'alcool pendant au moins les 5 semaines suivant le début de votre participation.
- De collaborer pleinement au bon déroulement de cette étude.
- De ne dissimuler aucune information relative à votre état de santé, aux médicaments que vous prenez ou aux symptômes que vous présentez.
- De ne participer à aucune autre étude clinique impliquant un traitement expérimental (médicament, dispositif médical ou procédure) pendant votre participation à cette étude.
- De porter en permanence sur vous la « carte d'urgence ». Cette précaution est indispensable pour garantir votre sécurité si vous deviez recevoir des soins d'urgence dans une institution qui ne vous connaît pas.

Contact

Si vous avez besoin d'informations supplémentaires, mais aussi en cas de problème ou d'inquiétude, vous pouvez contacter le médecin investigateur …… ou un membre de son équipe de recherche ……. au numéro de téléphone suivant (xx / xxx-xx-yy) ……..

En cas d'urgence ou en-dehors des heures de consultation, vous pouvez contacter le médiateur…… au numéro de téléphone suivant ……..., en mentionnant que vous participer à une étude clinique. Votre dossier contiendra les informations sur cette étude clinique, qui seront utiles pour le médecin de garde.

ÉTUDE HEP101

Étude Clinique multicentrique de phase II visant à évaluer la sécurité et l'efficacité préliminaire de 2 doses d'HepaStem chez des patients souffrant d'insuffisance hépatique aiguë sur une maladie chronique du foie.

Consentement éclairé du patient

ID du patient de l'étude : ……..

Participant

Je déclare que j'ai été informé(e) sur la nature de l'étude, son but, sa durée, les éventuels bénéfices et risques et ce que l'on attend de moi. J'ai pris connaissance du document d'information. J'ai eu suffisamment de temps pour y réfléchir et en parler avec une personne de mon choix, p. ex. mon médecin généraliste (MG) ou un membre de ma famille.

J'ai eu l'occasion de poser au médecin investigateur toutes les questions qui me sont venues à l'esprit et j'ai obtenu une réponse satisfaisante à mes questions.

Je comprends que ma participation à cette étude est volontaire et que je suis libre de mettre fin à ma participation à cette étude sans que cela ne modifie mes relations avec l'équipe thérapeutique en charge de ma santé.

Je comprends qu'au cours de cette étude clinique, mes données personnelles (en particulier mon dossier médical) seront récoltées, conservées et analysées. L'utilisation des informations relatives à ma santé est conforme aux dispositions légales et nécessite un consentement éclairé et volontaire préalable à ma participation à cette étude. Sans avoir signé le formulaire de consentement, je ne peux pas participer à l'étude.
Les données personnelles sont codées. J'ai le droit de consulter/corriger ces données.

Le consentement à la collecte et au traitement de mes données personnelles, en particulier des informations relatives à ma santé, est irrévocable. J'ai déjà été informé(e) que je peux mettre un terme à ma participation à cette étude à tout moment. Dans le cas où je retire mon consentement, je consens à ce que les données collectées jusqu'à ce moment-là puissent être analysées de manière pseudonyme (codées)

J'accepte que mes données soient conservées après la fin de l'étude ou mon interruption de l'étude, conformément aux exigences nationales requises.

J'accepte que les données d'étude collectées en vue d'atteindre les objectifs de cette étude puissent être traitées ultérieurement, pour autant que ce traitement soit limité au cadre de la présente étude pour une meilleure connaissance de la maladie et de son traitement.

J'accepte que le promoteur conserve des échantillons de matériel biologique récoltés en cours de l'étude après la fin de l'étude clinique, à des fins de recherches ultérieures mais limitées au cadre de la présente étude.

J'accepte que mon MG ou d'autres spécialistes en charge de ma santé soient informés de ma participation à cette étude clinique.

J'ai reçu une copie du document d'information du participant et du formulaire de consentement éclairé.

Prénom du patient : ……Nom du patient : ……

Signature : …… Date : ……

Témoin/interprète

J'ai été présent pendant l'entièreté du processus d'information du patient et je confirme que les informations relatives aux objectifs et procédures de l'étude ont été fournies de manière adéquate, que le participant (ou son représentant légal) a apparemment compris l'étude et que le consentement à participer à l'étude a été donné librement.

Prénom et nom du témoin/de l'interprète : ……..

Signature : …… Date : ……

Médecin investigateur

Je soussigné(e), ……., médecin investigateur, confirme avoir fourni verbalement les informations nécessaires sur l'étude et avoir fourni une copie du document d'information au participant.

Je confirme qu'aucune pression n'a été exercée pour persuader le patient d'accepter de participer à l'étude et que je suis prêt(e) à répondre à toutes les questions supplémentaires si cela s'avère nécessaire.

Je confirme travailler en accord avec les principes éthiques énoncés dans la dernière version de la « Déclaration d'Helsinki », des « Bonnes pratiques Cliniques » et de la loi nationale en vigueur relative aux expérimentations sur la personne humaine.

Prénom du médecin investigateur : ……Nom du médecin investigateur : ……

Signature : …… Date : ……

HEP101 STUDIE
MULTICENTRALE FASE II VEILIGHEIDSSTUDIE EN EFFECTIVITEIT-VOORSTUDIE VAN 2 DOSERINGSREGIMES VAN HEPASTEM BIJ PATIËNTEN MET ACUUT-OP-CHRONISCHE LEVERFALEN

Patiënteninformatie

Deze patiënteninformatie en dit toestemmingsformulier na informatie zijn bedoeld voor patiënten die lijden aan acuut-op-chronisch leverfalen die worden uitgenodigd om aan een wetenschappelijk onderzoek met HepaStem deel te nemen

Studiesponsor: **Promethera Biosciences**
Adres: **Rue Granbonpré 11**
1435 Mont St Guibert
België

Voornaamste onderzoeker: _____
Adres _____
Telefoonnummer: _____

Introductie

U wordt uitgenodigd om deel te nemen aan een klinische studie met het oog op de evaluatie van een medisch onderzoeksproduct voor de behandeling van uw ziekte. Een medisch onderzoeksproduct is een medisch product dat nog steeds wordt bestudeerd om de effectiviteit, veiligheid en werking ervan te evalueren.

De Onderzoeker (of de studiearts is de arts die verantwoordelijk is voor deze wetenschappelijke studie in het ziekenhuis) en de Sponsor (bedrijf dat het wetenschappelijk onderzoek start en financiert) hopen dat dit medisch product helpt bij de behandeling van patiënten met dezelfde ziekte als de uwe. Er is echter geen waarborg dat uw deelname aan deze studie uw gezondheid ten goede komt.

Vooraleer u instemt om aan deze studie deel te nemen, vragen we u om rekening te houden met de implicaties op het vlak van organisatie, mogelijke risico's en voordelen, wat u in staat moet stellen om, volledig bewust van de implicaties, een beslissing te nemen. Dit is gekend als het geven van een "geïnformeerde toestemming".

Gelieve deze informatie nauwkeurig te lezen en leg alle vragen waarmee u zit voor aan de onderzoeker of zijn/haar vertegenwoordiger. Dit document bestaat uit 2 delen: de informatie die essentieel is voor uw beslissing en, het ingevulde en ondertekende toestemmingsformulier.

Vooraleer u beslist, moet u bewust zijn van het volgende:

- Deze klinische studie wordt uitgevoerd nadat ze door een ethisch comité (in Leuven) werd beoordeeld na overleg met de ethische comités van andere deelnemende centra.
- Uw deelname is vrijwillig en moet verder zonder enige dwang verlopen. Een ondertekening van een document waarin u uw toestemming te kennen geeft, is vereist. Zelfs nadat u dit document hebt ondertekend, kunt u uw deelname stopzetten door de onderzoeker hierover te

informeren. Uw beslissing om niet deel te nemen zal geen impact hebben op de kwaliteit van de zorg of op uw relatie met de onderzoeker.

- De gegevens die in deze studie worden verzameld zijn vertrouwelijk en uw anonimiteit is altijd gewaarborgd, ook tijdens de publicatie van de resultaten.
- De sponsor heeft een polis getekend bij een verzekeringsmaatschappij, ingeval u enige schade zou oplopen in verband met uw deelname aan deze studie.

  Verzekeringsmaatschappij: QBE Syndicate

  Polisnummer: 16ME305419EA
- Uw deelname aan de studie is volledig gratis. U zult geen kosten aangerekend krijgen voor de onderzoeken die specifiek zijn voor deze studie. U zult echter ook niet betaald worden voor uw deelname aan deze studie.
- Uw onkosten voor uw vervoer naar het ziekenhuis (bv. de kosten voor uw auto, taxi, treinticket, enz.) zullen u vergoed worden met waardebonnen (1 waardebon/uitgevoerd onderzoeksbezoek na uw ontslag uit het ziekenhuis). Neem contact op met het studieteam voor praktische informatie.
- De sponsor zal het ziekenhuis/de onderzoeker vergoeden voor alle kosten voor bezoeken/consultaties, onderzoeken of behandelingen die specifiek zijn voor deze studie.
- U mag de onderzoeker contacteren of een lid van zijn/haar team, telkens wanneer u bijkomende informatie wenst.

Doelstellingen en beschrijving van het studieprotocol

Wij nodigen u uit om deel te nemen aan een klinische studie waarbij gebruik wordt gemaakt van menselijke levercellen (progenitorcellen genoemd) afkomstig van gedoneerde levers. Van deze cellen verwacht men dat ze een gecombineerd systemisch (in heel uw lichaam) en lokaal effect op de lever hebben. Men verwacht dat ze een immunomodulerende functie hebben, wat betekent dat ze de overdreven, ontstekingsreactie die bij uw ziekte werd vastgesteld helpen regelen en vooral een oplossing bieden voor het huidige acute event. De cellen die worden getest worden heterologe (van een andere persoon) progenitorcellen genoemd, afkomstig van de lever van een volwassen persoon (HHALPC) of HepaStem.

De cellen zijn stamcellen, die uit volwassen levers zijn geïsoleerd en ze zijn geen embryonale stamcellen. Ze worden op een specifieke wijze bewaard en zijn goedgekeurd door de gezondheidsinstanties om te worden gebruikt bij patiënten in het kader van een klinisch onderzoek.

Twaalf (12) patiënten zullen in Europa in deze studie worden opgenomen, waaronder ongeveer 6 in België. De onderzoeker zal aan patiënten voorstellen om deel te nemen, waarbij zoals bij u, een diagnose van levercirrose en acuut op chronisch leverfalen werd vastgesteld.

Het voornaamste doel van deze studie bestaat erin om de veiligheid en effectiviteit te beoordelen van twee (2) doseringen van HepaStem 4 maal toegediend in een periode van 14 dagen (om de 3 dagen 1 keer toegediend).

Deze studie is een open studie van 2 doseringsregimes. Dit betekent dat u ofwel

- 4 x een lage dosis cellen (250 miljoen cellen elk) in een periode van 14 dagen krijgt toegediend (dit betekent de toediening van 1 spuit van 50 ml tweemaal per week gedurende 2 weken) of

- 4 x een hoge dosis cellen (500 miljoen cellen in een periode van 14 dagen) (dit betekent de toediening van 2 spuiten van 50 ml tweemaal per week gedurende 2 weken)

In beide gevallen zullen u en uw onderzoeker op de hoogte zijn van de dosis die u zal worden toegediend.

Verloop van de studie

Na een behandeling van 2 weken met cellen en nog eens 2 weken tijdens de observatieperiode, zal u in de studie blijven tot 1 jaar voorbij is (follow-up periode). Binnen de eerste maand zal u 8 studiebeoordelingen krijgen, d.w.z. dagen met procedures en onderzoeken die specifiek aan de studie gekoppeld zijn. Tijdens de follow-up periode zal u 4 studiebeoordelingen krijgen.

De onderzoeken en procedures die in verband met de studie nodig zijn, worden hieronder beschreven. Enkele onderzoeken en procedures maken deel uit van de standaardzorg die in het ziekenhuis wordt verleend, terwijl anderen in het kader van de studie worden aangeboden.

Uw deelname aan de studie zal over 3 periodes verlopen:

1 Screeningsperiode: in die periode zal uw onderzoeker verifiëren of u in aanmerking komt om aan de studie deel te nemen en hij zal uw medische geschiedenis en de gegevens vergaren van enkele onderzoeken die werden gedaan, nadat u had toegestemd om aan de studie deel te nemen.

Er zullen ten hoogste 25 mL bloed (ongeveer 2 eetlepels bloed) worden afgenomen. Bloedstalen zijn reeds genomen in het kader van de zorgverlening in uw huidige klinische situatie; daarom zal geen bijkomende prik in uw arm worden gegeven om deze buisjes bloed te verzamelen.

Er zal ook een analyse van uw urine plaatsvinden.
De bloed- en urinestalen zullen ons toelaten om de parameters te analyseren die ons meer zullen zeggen over de staat van uw organen (lever, nieren, bloed), over uw virologische status (waaronder hepatitis en HIV) alsook uw immuniteit.
Indien het nog niet is gebeurd, zal een echografie worden genomen van uw lever en hart, X-stralen van uw borstkas en een electrocardiogram. Deze onderzoeken zijn niet-invasieve behandelingen en doen geen pijn.
Indien u tijdens uw opname in het ziekenhuis een leverbiopsie hebt gehad, zullen de resultaten van dit onderzoek worden verzameld.
U zal een “noodkaart” met informatie over deze studie ontvangen. Houd deze kaart steeds bij u. Indien u in het ziekenhuis wordt opgenomen, toon dan die kaart aan uw arts en informeer hem/haar dat u aan deze studie deelneemt.

Na de screeningsperiode, zal u, nadat aan alle criteria is voldaan om aan deze studie deel te nemen, in staat zijn om de actieve periode in de studie aan te vangen en HepaStem te krijgen.

2 Actieve periode: Tijdens deze periode zal u in de loop van 14 dagen 4 x HepaStem toegediend krijgen. Afhankelijk van de groep waartoe u behoort, zal u 250 of 500 miljoen cellen worden toegediend. Dit beantwoordt aan een volume van 50 of 100 mL vloeistof.

HepaStem zal intraveneus worden toegediend, wat betekent dat een naald in een kleine of grote ader zal worden geplaatst om het product toe te dienen. Voorafgaand aan de HepaStem infusie zal medicatie worden gegeven om slechte reacties van het lichaam op het product te voorkomen.

Tijdens het toedienen en in elk geval 2, 3 en 4 weken na de eerste toediening, zal u klinische onderzoeken ondergaan en zullen er bloedstalen worden genomen (max 15mL bloed, ongeveer 1 eetlepels bloed).
De bloedstalen zullen toelaten om informatie in te winnen over de status van uw organen (lever, nieren, bloed).
Er zal een echografie van uw hart worden gemaakt na de eerste toediening en een echografie van uw lever voor elke infusie, 2 en 4 weken na de eerste toediening.

3_ Follow-up periode: tijdens deze periode, zullen we u vragen om 2 maanden, 3 maanden, 6 maanden en 12 maanden na de eerste celinfusie naar het ziekenhuis terug te komen. Bij deze bezoeken zal u een klinisch onderzoek ondergaan en een bloedstaal (max 15mL bloed, ongeveer 1 eetlepels bloed). De resultaten van deze onderzoeken zullen worden verzameld.
De bloedstalen zullen ons toelaten om de parameters te analyseren die ons informatie zullen schenken over de status van uw organen (lever, nieren, bloed).
Indien u tijdens die periode een levertransplantatie hebt, zal indien mogelijk, een staal van uw verwijderde lever worden verzameld.

Na de follow-up periode, zullen we u vragen of u wilt instemmen om deel uit te maken van ons registratiesysteem voor bijkomende en regelmatige follow-up.

Risico's en ongemakken

A: Bijwerkingen van het medicijn van deze studie

- Alle medicijnen hebben gekende of onvoorspelbare bijwerkingen. Ook wanneer een voorafgaande studie heeft aangetoond dat HepaStem goed werd verdragen, kunt u toch de volgende bijwerkingen ervaren: **op korte termijn:**
  - trombose
- Ademhalingsmoeilijkheden overgevoeligheidsreactie of een reactie op de infusie: dit treedt op wanneer het immuunsysteem van het lichaam overmatig reageert op iets als medicatie. Overgevoeligheidsreacties kunnen omvatten: huidirritatie, roodheid, jeuk, zwelling, vochtafscheiding, korstvorming, huiduitslag, erupties, hoesten of kortademigheid, schorre stem, hoofdpijn, verstopte of lopende neus, niezen, rode (bloeddoorlopen) ogen, maagpijn, misselijkheid, braken, diarree, vermoeidheid, keelpijn, duizeligheid. Deze reacties kunnen schadelijk, ongemakkelijk of in sommige gevallen dodelijk zijn (in geval van anafylaxie): **op middellange of lange termijn:**
  - verspreiding in verschillende organen waar cellen de ontwikkeling van tumoren in de hand kunnen werken, hoewel dergelijke events zelden i.v.m. celtherapie werden gerapporteerd, een immuniteitsreactie aangezien HepaStem bestaat uit cellen van een andere persoon, wat eventueel tot afstoting van cellen kan leiden.

Andere risico's en ongemakken die voor het ogenblik niet bekend zijn, zouden zich kunnen voordoen. Het is daarom heel belangrijk dat elk nieuw gezondheidsprobleem vlug aan de onderzoeker wordt gerapporteerd, ongeacht of u al dan niet denkt dat het met de studie te maken heeft.

C: Risico's in verband met procedures die specifiek zijn voor deze studie

Er zijn ook risico's/ ongemakken, die verband houden met specifieke onderzoeken die in verband met deze studie zullen worden uitgevoerd:

- De intraveneuze toediening kan het volgende veroorzaken:

  - Pijn, zoals voor elke injectie.
  - Infectie. Elke opening in de huid houdt een risico van infectie in, hoewel intraveneuze injectie een aseptische procedure is.
  - Flebitis: ontsteking van een ader, die door infectie kan worden veroorzaakt.
  - Infiltratie: infiltratie doet zich voor wanneer een intraveneuze vloeistof of medicatie per toeval in het omgevende weefsel eerder dan in de ader terechtkomt.
  - Embolie: een bloedklonter of vaste massa, zoals ook een luchtbel, kan door een IV in de bloedsomloop terechtkomen en een bloedvat blokkeren. Evenwel, het is bijna onmogelijk om op een gevaarlijke manier lucht door een perifere IV te injecteren. Het risico is groter met een centrale IV.
- Het **afnemen van bloed,** noodzakelijk, voor een analyse kan (zelden) pijn veroorzaken, bloeden, blauwe plekken of infectie bij de plaats van de injectie tot gevolg hebben. Ook kunnen patiënten zich tijdens de procedure duizelig voelen of ze kunnen flauw vallen.

Het personeel dat bloed afneemt, zal de beste medische zorgen toedienen om ongemakken te voorkomen of ze tot een minimum te herleiden.

Bekendmaking van nieuwe informatie

Het kan gebeuren dat belangrijke nieuwe informatie bij het onderzoek m.b.t. HepaStem ter beschikking komt te staan. U zal op tijd worden geïnformeerd over elk nieuw element dat uw beslissing kan beïnvloeden om verder aan deze studie deel te nemen.

Indien u, in het licht van de nieuwe informatie, beslist om verder aan de studie deel te nemen, zal uw onderzoeker ervoor zorgen dat u verder de beste behandeling krijgt.

Contraceptie, zwangerschap en borstvoeding

Vrouwelijke deelnemer**:** omdat de gevolgen van HepaStem op een ongeboren kind of zuigeling niet echt gekend zijn, zal u niet worden toegelaten om aan deze klinische studie deel te nemen, wanneer u zwanger bent, wenst zwanger te worden of indien u borstvoeding geeft.

Indien u aan deze studie wenst deel te nemen, moet u één van de goedgekeurde contraceptiemethodes gebruiken (zodat u niet zwanger wordt). Uw arts zal de verschillende geschikte opties met u bespreken.

Baten

Indien u akkoord gaat om aan deze studie deel te nemen, kan HepaStem al dan niet blijken een gunstige werking te hebben bij de behandeling van uw ziekte of bij het verlichten van de symptomen, vooral bij het helpen oplossen van de huidige acute decompensatie van de leverfunctie.

De informatie die men dankzij deze studie krijgt, kan ertoe bijdragen om een betere kennis te krijgen van het gebruik van dit medisch product of van de ontwikkeling van een nieuw medisch product voor de behandeling van acuut-op chronisch leverfalen bij toekomstige patiënten.

Alternatieve behandeling:

Geen enkele door de overheden goedgekeurde behandeling van uw toestand, is voor het ogenblik in Europa beschikbaar.

De enige behandeling die nu beschikbaar is, is levertransplantatie. Evenwel, deze behandeling is ver van ideaal, wegens gebrek aan transplantatieorganen.

Terugtrekking uit de studie

Uw deelname is vrijwillig en u hebt het recht om u te allen tijde uit de studie terug te trekken, zonder dat u een reden opgeeft. Evenwel, het kan voor de onderzoeker en de sponsor van de studie nuttig zijn om te weten of u zich terugtrekt wegens de te grote last veroorzaakt door de behandeling (te veel onaangename bijwerkingen, bijvoorbeeld).

Het is ook mogelijk dat de onderzoeker u uit de studie haalt, omdat u zwanger bent, omdat hij/zij denkt dat het beter is voor uw gezondheid of omdat hij/zij er achter is gekomen dat u de instructies die aan de deelnemers werden gegeven niet opvolgt.

Tenslotte kunnen de bevoegde nationale of internationale overheden, het ethisch comité dat de studie eerst goedkeurde of de sponsor beslissen om de studie stop te zetten.

Als u uw toestemming om deel te nemen aan de studie intrekt, dan zullen, om de geldigheid van het onderzoek te garanderen, de gecodeerde gegevens tot het punt waarop u zich terugtrok, bewaard blijven. Er mogen geen nieuwe gegevens naar de sponsor verzonden worden.

Als u uw goedkeuring om deel te nemen aan de studie intrekt, kunt u contact opnemen met de onderzoeker en vragen om de stalen die nog niet gebruikt zijn, te laten vernietigen. De resultaten verkregen uit uw stalen voordat u uw goedkeuring introk, blijven eigendom van de studiesponsor.

Behandeling nadat de studie werd stopgezet

In al deze situaties van terugtrekking uit de studie, maar ook wanneer de geplande periode van uw deelname is beëindigd, zal uw onderzoeker de staat van uw gezondheid onderzoeken en de best beschikbare behandeling voorschrijven.

Stalen van biologisch materiaal dat gedurende de studie werd verzameld

De sponsor van deze studie verzekert dat de stalen (zoals bloed, urine, weefsel van de lever) alleen in het kader van de studie zullen worden gebruikt.

- De procedure voor het coderen van stalen is dezelfde als deze die wordt gebruikt voor uw medische gegevens. Stalen die naar de sponsor worden verstuurd, zullen daarom alleen uw studie-ID-code bevatten.
- De beheerder van deze stalen (het hematologielaboratorium van de Cliniques St. Luc in Brussel en het Translational Research Center, KU Leuven, Leuven) zal ze gebruiken binnen de context van klinisch onderzoek en ze vernietigen aan het eind van de geplande bewaarperiode.
- Het staal van afgenomen biologisch materiaal wordt beschouwd als een ‘donatie’ en u moet er zich van bewust zijn dat u, in principe, geen enkel financieel voordeel (royalty’s) zult ontvangen geassocieerd met de ontwikkeling van nieuwe therapieën die zijn afgeleid van het gebruik van uw donatie van biologisch materiaal en die mogelijk commerciële waarde kunnen hebben.

Het overschot van stalen zal worden vernietigd zodra de analyses die in dit document zijn beschreven, werden uitgevoerd (ten laatste, één jaar na beëindiging van de studie)

Vertrouwelijkheid en gegevensbescherming

Uw deelname aan de studie betekent dat u ermee akkoord gaat dat de onderzoeker gegevens over u verzamelt en dat de studiesponsor deze gegevens gebruikt voor onderzoeksdoeleinden en in verband met wetenschappelijke en medische publicaties.

U hebt het recht om de onderzoeker te vragen welke gegevens er over u verzameld worden en waarvoor ze in functie van de studie gebruikt worden. Deze gegevens omvatten uw huidige klinische situatie, maar ook wat achtergrondgegevens, de resultaten van onderzoeken uitgevoerd binnen de

context van uw gezondheidszorg in overeenstemming met de huidige standaarden en natuurlijk ook de resultaten van onderzoeken vereist door het protocol. U hebt het recht om deze gegevens te inspecteren en verbeteren als ze fout zouden zijn. Deze rechten zijn gegarandeerd door de wet van 8 december 1992 op de bescherming van de persoonlijke levenssfeer in functie van het verwerken van persoonlijke gegevens en door de wet van 22 augustus 2002 over de rechten van de patiënt.

De onderzoeker is gebonden door het beroepsgeheim over wat de verzamelde gegevens betreft.

Dit betekent dat hij/zij verplicht is om nooit uw naam te vermelden in de context van een publicatie of conferentie en dat hij/zij ook uw gegevens zal coderen (uw identiteit zal vervangen worden door een ID-code in de studie) alvorens ze naar de beheerder van de database van verzamelde gegevens te versturen (Clinical Department, Promethera Biosciences).

De onderzoeker en zijn/haar team zullen daarom de enigen zijn die een link kunnen maken tussen de gegevens die tijdens de studie verzonden worden en uw medisch dossier. Voor deze studie vereist de wet dat deze link met uw dossier gedurende minstens 30 jaar en maximaal 50 jaar bewaard blijft, in overeenstemming met de Belgische wet van 19 december 2008 over het gebruik van menselijk biologisch materiaal en de van toepassing zijnde koninklijke besluiten. De persoonlijke gegevens die verstuurd worden, zullen geen combinatie van elementen bevatten waaruit u geïdentificeerd kunt worden.

Voor de beheerder van de studiegegevens die wordt aangeduid door de sponsor, zullen de verstuurde gegevens het niet mogelijk maken om u te identificeren. De beheerder is verantwoordelijk voor het verzamelen van de gegevens van alle onderzoekers die deelnemen aan deze studie en ze te verwerken en beschermen in overeenstemming met de vereisten van de Belgische wet op de bescherming van de privacy.

Om de kwaliteit van de studie te verifiëren, is het mogelijk dat uw medisch dossier onderzocht wordt door personen die gebonden zijn door het beroepsgeheim en aangewezen door het ethisch comité, de sponsor van de studie of een onafhankelijk controle-orgaan. In elk geval mag dit onderzoek van uw medisch dossier alleen plaatsvinden onder de verantwoordelijkheid van de onderzoeker en onder toezicht van één van de door hem/haar aangeduide medewerkers.

De (gecodeerde) studiegegevens zullen mogelijk verstuurd worden naar Belgische of andere regelgevende autoriteiten, de relevante ethische comités, andere artsen en/of organisaties die samenwerken met de sponsor.

Ze zullen mogelijk ook verstuurd worden naar andere vestigingen van de sponsor in België en in andere landen waar de standaarden voor het beschermen van persoonlijke gegevens mogelijk anders of minder streng zijn. Zoals hierboven uitgelegd, worden de verstuurde gegevens gecodeerd. De sponsor verbindt zich ertoe de beperkingen van de Europese Richtlijn en de Belgische wetgeving op de bescherming van de privacy te respecteren.

Uw toestemming om deel te nemen aan deze studie impliceert hierdoor uw toestemming voor het gebruik van uw gecodeerde medische gegevens voor het doel beschreven in dit informatieformulier en voor de overdracht ervan naar de hierboven vermelde personen en autoriteiten.

De sponsor verbindt zich ertoe om de gegevens alleen te gebruiken binnen de context van de studie waaraan u deelneemt.

Verzekering

Elke deelname aan een klinische studie houdt een risico in, hoe klein ook. Zelfs zonder fout aanvaardt de sponsor verantwoordelijkheid voor schade die aan de deelnemer wordt veroorzaakt (of in het geval van overlijden, zijn/haar rechthebbenden) en die rechtstreeks of onrechtstreeks

verbonden is met zijn/haar deelname aan de studie. De sponsor heeft een verzekering afgesloten voor deze verantwoordelijkheid.

We vragen u daarom om elk nieuwe gezondheidsprobleem te melden aan de onderzoeker voordat u een andere arts raadpleegt, andere medicatie begint in te nemen of een andere medische behandeling ondergaat. Als u, om eender welke reden, een andere arts raadpleegt tijdens deze klinische studie, moet u hem/haar inlichten dat u deelneemt aan een klinische studie/uw deelnemerskaart van de klinische studie tonen. Dit kan belangrijk zijn voor het stellen van een diagnose en het behandelen van uw klachten.

Als de onderzoeker denkt dat een verband met de studie mogelijk is (de verzekering dekt het natuurlijke verloop van uw ziekte of de bekende bijwerkingen van uw normale behandeling niet), zal hij/zij de studiesponsor informeren, die de aangifteprocedure bij de verzekeringsmaatschappij zal opstarten. De laatste zal een deskundige aanstellen (als dit nodig wordt geacht) om te beoordelen of er een verband bestaat tussen uw nieuwe gezondheidsproblemen en de studie.

In geval van onenigheid met de onderzoeker of met de door de verzekeringsmaatschappij aangestelde deskundige en ook wanneer u dat nodig acht, kunt u, of kunnen uw nabestaanden in geval van overlijden, de verzekeraar rechtstreeks in België dagvaarden (naam van verzekeringsmaatschappij, polisnummer, contactpersoon).

De wet voorziet dat de verzekeraar voor de rechtbank moet verschijnen van de locatie waar het schadegeval optrad, voor de rechtbank van uw woonplaats, of voor de rechtbank van de hoofdzetel van de verzekeraar.

Indien u aan deze klinische studie deelneemt, verzoeken wij om:

- Ten minste 5 weken voor het begin van uw deelname geen alcohol te drinken.
- Volledig mee te werken aan een rimpelloos verloop van de studie.
- Geen enkele informatie in verband met uw gezondheidstoestand, de medicatie die u neemt of de symptomen die u ondervindt, te verbergen.
- Niet deel te nemen aan een andere klinische studie waarbij er sprake is van een behandeling in het kader van het onderzoek, of het nu gaat om een medisch hulpmiddel of een procedure, terwijl men aan deze studie deelneemt.
- De "noodkaart" steeds bij u te hebben. Dit is absoluut noodzakelijk voor uw veiligheid in geval u dringende zorg nodig hebt in een instelling die u niet kent.

Contact

Indien u verder informatie nodig hebt, maar ook indien u problemen of zorgen heeft, kunt u de onderzoeker ……of een lid van zijn/haar research team..... op het volgende telefoonnummer (xx / xxx-xx-yy).....contacteren.

In noodgeval of buiten de consultatie-uren, contacteer de ombudsman …… op het volgend telefoonnummer ……... en vermeld dat u aan een klinische studie deelneemt. Uw dossier zal informatie bevatten die nuttig is voor de dienstdoende arts in verband met deze klinische studie.

HEP101 STUDIE

MULTICENTRALE FASE II VEILIGHEIDSSTUDIE EN EFFECTIVITEIT-VOORSTUDIE VAN 2 DOSERINGSREGIMES VAN HEPASTEM BIJ PATIËNTEN MET ACUUT-OP-CHRONISCH LEVERFALEN

Toestemming van de geïnformeerde patiënt

ID van de patiënt in de studie: ……..

Deelnemer

Ik verklaar dat ik ingelicht ben over de aard van de studie, haar doelstelling, duur, risico's en baten, alsook over wat van mij wordt verwacht. Ik heb kennis genomen van het informatiedocument en ik heb voldoende tijd gehad om erover na te denken en te bespreken met een persoon van mijn keuze, zoals mijn huisarts of een familielid.

Ik heb de gelegenheid gehad om aan de onderzoeker de vragen te stellen die bij me opkwamen en ik heb een bevredigend antwoord op mijn vragen gekregen.

Ik begrijp dat mijn deelname aan deze studie vrijwillig is en dat ik vrij ben om mijn deelname aan deze studie te beëindigen zonder dat mijn relatie met het therapeutisch team dat verantwoordelijk is voor mijn gezondheid, in het gedrang komt.

Ik ben er me van bewust dat voor deze klinische studie mijn persoonlijke gegevens, vooral mijn medische gegevens worden verzameld, opgeslagen en geanalyseerd. Het gebruik van de informatie in verband met mijn gezondheid stemt overeen met de wettelijke bepalingen en vereist, voorafgaand aan deze studie een vrijwillig gegeven geïnformeerde toestemming. Zonder daaropvolgende toestemming kan ik niet aan de studie deelnemen.

De persoonlijke gegevens zijn geëncrypteerd. Ik heb het recht mijn gegevens te bekijken/te verbeteren.

De toestemming om mijn persoonlijke gegevens te verzamelen en te verwerken, vooral informatie over mijn gezondheid, is onherroeppelijk. Ik ben reeds geïnformeerd dat ik mijn deelname aan dit onderzoek te allen tijde kan beëindigen. Ingeval van een dergelijke terugtrekking van mijn toestemming, stem ik ermee in dat mijn gegevens die tot hiertoe zijn verzameld, onder pseudoniem (geëncrypteerd) kunnen worden geanalyseerd.

Ik stem ermee in dat mijn gegevens na beëindiging van de studie of bij onderbreking zullen worden opgeslagen volgens de nationale vereisten.

Ik stem ermee in dat de gegevens van deze studie, verzameld voor de doelstellingen van deze studie, op een later datum worden verwerkt, op voorwaarde dat de verwerking uitsluitend verband houdt met het kader van de huidige studie voor een beter begrip van de ziekte en haar behandeling.

Ik stem ermee in dat de sponsor stalen behoudt van het biologisch materiaal dat tijdens de studie, op het einde van het klinisch onderzoek voor verdere onderzoeksdoeleinden werd verzameld, maar binnen het kader van de huidige studie.

Ik stem ermee in dat mijn huisarts of andere specialisten die verantwoordelijk zijn voor mijn gezondheid, worden geïnformeerd over mijn deelname aan deze klinische studie.

Ik heb een kopie ontvangen van de informatie voor de deelnemer en een geïnformeerd toestemmingsformulier.

Achternaam van de patiënt: ……Voornaam: ……

Handtekening: …… Datum: ……

Getuige/Tolk

Ik was aanwezig tijdens het volledige proces van de patiënteninformatie en ik bevestig dat de informatie over de doelstellingen en de procedures van de studie naar behoren werd verleend, dat de deelnemer (of zijn/haar vertegenwoordiger) blijkbaar de studie begreep en dat de toestemming om deel te nemen in volle vrijheid werd gegeven.

De getuige/tolk: … Achternaam & Voornaam: ……..

Handtekening: …… Datum : ……

De onderzoeker

Ik, de ondergetekende,……., onderzoeker, bevestig dat ik reeds mondeling de nodige informatie over de studie heb verstrekt en dat ik aan de deelnemer een kopie van het informatief document heb gegeven.

Ik bevestig dat er geen druk werd uitgeoefend om de patiënt te overtuigen om zijn toestemming te geven om aan deze studie deel te nemen en dat ik, zo nodig, bijkomende vragen wil beantwoorden.

Ik bevestig dat ik in overeenstemming met ethische principes handel die zijn opgenomen in de laatste versie van de “Verklaring van Helsinki”, de “Goede Klinische Praktijk” en de huidige nationale regeling, in verband met onderzoeken waaraan mensen deelnemen.

Achternaam van de onderzoeker: ……Voornaam……

Handtekening: …… Datum: ……

ÉTUDE HEP101

ÉTUDE CLINIQUE MULTICENTRIQUE DE PHASE II VISANT A EVALUER LA SECURITE ET L'EFFICACITE PRELIMINAIRE DE 2 DOSES D'HEPASTEM CHEZ DES PATIENTS SOUFFRANT D'INSUFFISANCE HEPATIQUE AIGUË SUR UNE MALADIE CHRONIQUE DU FOIE.

Formulaire d'information pour le patient

Ce formulaire d'information pour le patient et le formulaire de consentement éclairé sont destinés aux patients souffrant d'une insuffisance hépatique aiguë sur maladie chronique du foie (ACLF) ou aux représentants légaux de ces patients qui sont invités à participer à une étude de recherche clinique réalisée avec HepaStem

Promoteur de l'étude : **Promethera Biosciences**
Adresse : **Rue Granbonpré 11**
1435 Mont St Guibert
Belgique

Investigateur principal : _____
Adresse : _____
Numéro de téléphone : _____

Introduction

Vous êtes invité(e) à participer à une étude clinique destinée à évaluer un médicament expérimental pour le traitement de votre maladie. Un médicament expérimental est un médicament faisant encore l'objet d'études pour évaluer son efficacité, sa sécurité d'emploi ou son mécanisme d'action.

À l'attention du patient : Il peut arriver qu'au moment de votre inclusion dans l'étude, vous n'étiez pas pleinement capable de décider vous-même si vous souhaitiez participer on non à cette étude. Il est alors habituel d'utiliser un représentant légal, à qui l'on demande de prendre une décision sur la participation éventuelle d'une personne à l'étude, en tenant compte de ses intérêts et de sa probable volonté. Votre représentant légal a accepté que vous participiez à cette étude, sachant que, dès que votre situation clinique le permettrait, vous seriez informé(e) de votre participation à une étude clinique, et que vous seriez à ce moment libre de décider de poursuivre votre participation ou de l'interrompre. Nous vous demandons à présent de confirmer votre souhait de poursuivre cette participation ou de l'interrompre.

À l'attention du représentant légal : En raison de sa situation clinique, on estime que la personne que vous représentez est actuellement incapable de décider si elle souhaite ou non participer à cette étude, en ayant une pleine conscience des implications liées à l'étude. Vous êtes dès lors invité(e) à décider si la personne que vous représentez doit participer ou non à cette étude clinique, en tenant compte de ses intérêts et de sa probable volonté.

Dans la suite de ce document, le texte est rédigé comme s'il s'adressait directement à la personne que vous représentez.

L'investigateur (ou médecin de l'étude, c.-à-d. le médecin responsable de cette étude de recherche à l'hôpital) et le promoteur (la firme qui initie et finance cette recherche) espèrent que ce médicament expérimental peut présenter des avantages pour le traitement de patients atteints de la même maladie que la vôtre. Néanmoins, rien ne garantit que vous tiriez un bénéfice de votre participation à cette étude.

Avant que vous n'acceptiez de participer à cette étude, nous vous invitons à prendre connaissance de ses implications en termes d'organisation, risques et bénéfices éventuels, afin que vous puissiez prendre une décision en toute connaissance de cause. C'est ce qu'on appelle donner un « consentement éclairé ».

Veuillez lire attentivement ces quelques pages d'information et poser toutes les questions que vous souhaitez à l'investigateur ou à la personne qui le représente. Ce document comprend 2 parties : l'information essentielle à votre prise de décision et votre formulaire de consentement écrit.

Avant de prendre une décision, vous devez savoir que :

- Cette étude clinique est mise en œuvre après avoir fait l'objet d'une évaluation par un comité d'éthique (de Louvain) après consultation des comités d'éthique d'autres centres participants.
- Votre participation est volontaire et doit rester libre de toute contrainte. Elle nécessite la signature d'un document exprimant votre consentement. Même après avoir signé ce document, vous pouvez interrompre votre participation en informant le médecin investigateur. Votre décision de ne pas ou de ne plus participer à l'étude n'aura aucun impact sur la qualité de vos soins ni sur vos relations avec le médecin investigateur.
- Les données recueillies au cours de l'étude sont confidentielles. Votre anonymat sera toujours garanti, y compris lors de la publication des résultats.
- Le promoteur a souscrit une assurance au cas où vous subiriez un dommage lié à votre participation à cette étude clinique.

  Compagnie d'assurances : QBE Syndicate
  Numéro de la police : 16ME305419EA
- Votre participation à cette étude est totalement gratuite. Aucun frais ne vous sera facturé pour les examens spécifiques liés à cette étude. Cependant, vous ne serez pas rémunéré(e) pour votre participation à cette étude.
- Les dépenses liées à votre transport jusqu'à l'hôpital (comme les frais de voiture, de taxi, le ticket de train, etc.) vous seront remboursées avec des vouchers (1 pièce/visite de l'étude effectuée après votre sortie de l'hôpital). Veuillez contacter le personnel de l'étude pour les détails pratiques.
- Le promoteur remboursera l'hôpital/médecin investigateur pour tous les frais de visites/consultations, examens et traitements spécifiques à l'étude.
- Vous pouvez contacter le médecin investigateur ou un membre de son équipe à tout moment si vous avez besoin d'informations complémentaires.

Objectifs et description du protocole de l'étude

Vous êtes invité(e) à participer à une étude clinique portant sur le traitement par des cellules de foie humain (appelées « cellules progénitrices ») provenant de foies de donneurs. On espère que ces cellules ont un effet combiné systémique (dans tout le corps) et local dans le foie. Nous pensons qu'elles jouent un rôle immunomodulateur, c.-à-d. qu'elles aident à réguler la réponse inflammatoire exagérée qui est observée au cours de votre maladie, en particulier qu'elles aident à résoudre l'épisode aigu actuel de votre affection. Les cellules testées sont appelées « cellules progénitrices allogéniques (c.-à-d. provenant d'une autre personne) isolées à partir de foie humain adulte » ou HepaStem.
Les cellules sont des cellules souches isolées à partir de foies adultes et ne sont pas des cellules souches embryonnaires. Elles sont conservées selon une méthode spécifique et les autorités de santé ont approuvé leur utilisation chez des patients dans le cadre d'une étude clinique.

Douze (12) patients seront inclus dans cette étude en Europe, dont environ 6 en Belgique. Le médecin investigateur proposera à des patients qui, comme vous, présentent un diagnostic de cirrhose et une insuffisance hépatique aiguë sur maladie chronique du foie, de participer à cette étude.

L'objectif principal de cette étude est d'évaluer la sécurité et l'efficacité de deux (2) posologies d'HepaStem administrées 4 fois sur une période de 14 jours (1 administration tous les 3 jours).

Il s'agit d'une étude réalisée en ouvert pour évaluer 2 schémas posologiques, ce qui signifie que vous recevrez

- 4 administrations de la dose faible de cellules (250 millions de cellules pour chaque administration) sur une période de 14 jours (cela signifie l'administration d'1 seringue de 50 ml deux fois par semaine pendant 2 semaines), ou
- 4 administrations de la dose élevée de cellules (500 millions de cellules pour chaque administration) sur une période de 14 jours (cela signifie l'administration de 2 seringues de 50 ml deux fois par semaine pendant 2 semaines).

Dans les deux cas, vous et votre médecin investigateur saurez quelle posologie vous recevez.

Déroulement de l'étude

Après la phase de traitement de 2 semaines par les cellules et une période supplémentaire de 2 semaines de surveillance, vous resterez encore dans l'étude pendant 1 an (phase de suivi). Au cours du premier mois, vous ferez l'objet de 8 évaluations d'étude, c.-à-d. que vous subirez ces jours-là des procédures et des examens spécifiques à l'étude. Pendant la phase de suivi, vous ferez l'objet de 4 évaluations d'étude.

Les examens et procédures nécessaires liés à l'étude sont décrits ci-dessous. Certains de ces examens et procédures font partie des soins standards prodigués par votre hôpital, tandis que d'autres sont offerts dans le cadre de cette étude.

Votre participation à l'étude sera constituée de 3 phases :

1 Phase de sélection : pendant cette phase, votre médecin investigateur vérifiera si vous êtes éligible pour participer à cette étude, et établira un relevé de vos antécédents médicaux et des résultats de certains examens réalisés après avoir reçu votre accord pour participer à l'étude.

Tout au plus 25 ml supplémentaires de sang (environ 2 cuillères à soupe) seront prélevés. Des prises de sang sont déjà réalisées dans le cadre des soins liés à votre situation clinique actuelle ; aucune piqûre supplémentaire ne sera donc réalisée sur votre bras pour prélever ces tubes de sang.

Certaines analyses d'urine seront également réalisées.

Les échantillons de sang et d'urine permettront d'analyser des paramètres qui fourniront des informations sur l'état de vos organes (foie, reins, sang), sur votre statut virologique (notamment pour l'hépatite et l'infection à VIH) et sur votre immunité.

Si ce n'est pas déjà fait, on réalisera une échographie de votre foie et de votre cœur, une radiographie du thorax et un électrocardiogramme. Ces examens ne sont pas invasifs et ils sont indolores.

Si vous avez déjà subi une biopsie du foie pendant cette hospitalisation, les résultats de cet examen seront collectés.

Vous recevrez une « carte d'urgence » mentionnant toutes les informations sur l'étude. Veuillez porter cette carte sur vous en permanence. Si vous n'êtes pas hospitalisé(e), veuillez montrer cette carte à votre médecin et l'informer que vous participez à cette étude.

Après la phase de sélection, si vous répondez à tous les critères nécessaires pour entrer dans l'étude, vous serez en mesure de débuter la phase active de l'étude et vous recevrez le médicament HepaStem.

2_ Phase active de l'étude : Pendant cette phase, vous recevrez 4 administrations d'HepaStem sur une période de 14 jours. En fonction du groupe auquel vous appartenez, vous recevrez 250 ou 500 millions de cellules par administration, ce qui équivaut à un volume de 50 ou 100 ml de liquide.

HepaStem sera administré par voie intraveineuse, ce qui signifie qu'une aiguille sera introduite dans une veine de petit ou grand calibre pour administrer le médicament. En vue de prévenir les réactions indésirables de votre corps au médicament, un médicament sera administré avant la perfusion d'HepaStem.

Pendant chaque administration et dans tous les cas, 2, 3 et 4 semaines après la 1ère administration, vous ferez l'objet d'examens cliniques et des échantillons de sang seront prélevés (environ 15 mL de sang soit 1 cuillère à soupe). Les échantillons de sang permettront d'obtenir des informations sur l'état de vos organes (foie, reins, sang).
Une échographie de votre cœur sera réalisée après la 1ère administration et une échographie de votre foie sera réalisée après avant chaque perfusion, puis 2 et 4 semaines après la 1ère administration.

3_ Phase de suivi : pendant cette période, vous serez invité(e) à revenir à l'hôpital 2 mois, 3 mois, 6 mois et 12 mois après l'administration de la 1ère perfusion de cellules. Au cours de ces visites, vous ferez l'objet d'un examen clinique et un échantillon de sang sera prélevé (environ 15 mL de sang soit 1 cuillère à soupe). Les résultats de ces examens seront collectés.
Les échantillons de sang permettront d'analyser des paramètres qui fourniront des informations sur l'état de vos organes (foie, reins, sang).
Pendant cette période, si vous avez subi une transplantation du foie, un échantillon de votre foie explanté sera collecté si possible.

Après la phase de suivi, nous vous demanderons si vous acceptez d'être inscrit(e) dans notre registre dans le cadre d'un suivi régulier et complémentaire.

Risques et inconvénients

A : Effets secondaires du médicament de l'étude

- Tous les médicaments ont des effets secondaires connus ou imprévisibles. Même si l'étude précédente a révélé qu'HepaStem était bien toléré, il est toujours possible que vous présentiez les effets secondaires suivants : **à court terme :**
  - Thrombose
- Affection respiratoire Réaction d'hypersensibilité ou réaction à la perfusion : ceci se produit lorsque le système immunitaire du corps sur-réagit à quelque chose comme un médicament. La réaction d'hypersensibilité peut comprendre : irritation de la peau, rougeur, démangeaisons, gonflement, suintement, formation de croûtes, éruptions, toux ou essoufflement, enrouement de la voix, maux de tête, nez bouché ou qui coule, éternuements, yeux rouges, douleurs à l'estomac, nausée, vomissements, diarrhée, fatigue, mal de gorge, vertiges. Ces réactions peuvent être préjudiciables, inconfortables ou occasionnellement, fatales (en cas d'anaphylaxie). **à moyen ou long terme :**
  - distribution dans différents organes où les cellules peuvent favoriser le développement d'une tumeur, même si ces effets ont été rarement rapportés avec la thérapie cellulaire réaction immunitaire car HepaStem est constitué de cellules provenant d'une autre personne, ce qui peut éventuellement induire une réaction de rejet des cellules.

D'autres risques et inconvénients inconnus à ce jour pourraient également apparaitre. Il est donc très important de signaler rapidement tout nouveau problème de santé au médecin investigateur, que vous pensiez ou non qu'il soit en rapport avec l'étude.

C : Risques associés aux procédures spécifiques à l'étude

Il existe également des risques/inconvénients associés aux examens spécifiques qui seront réalisés dans le cadre de cette étude :

- L'administration intraveineuse peut provoquer les effets suivants :
  - Douleur, comme pour toute injection.
  - Infection. Toute ouverture au niveau de la peau peut comporter un risque d'infection, même si l'insertion intraveineuse est une procédure aseptique.
  - Phlébite : inflammation d'une veine pouvant être causée par une infection.
  - Infiltration : une infiltration survient lorsqu'un liquide ou un médicament administré par voie intraveineuse pénètre accidentellement dans le tissu environnant plutôt que dans la veine.
  - Embolie : un caillot sanguin ou une autre particule solide, ou encore une bulle d'air, peut être introduit dans la circulation au cours de l'administration IV et bloquer finalement un vaisseau. Cependant, il est presque impossible d'injecter de l'air au cours d'une administration IV par voie périphérique. Le risque est plus élevé en cas d'administration IV par voie centrale.
- La **prise de sang** nécessaire aux analyses peut (rarement) causer une douleur, un saignement, une contusion (bleu) ou une infection autour du site d'injection. Chez certains patients, des étourdissements, ou même un évanouissement, peuvent survenir pendant la procédure.

L'équipe qui réalisera la prise de sang fera usage des meilleurs soins médicaux pour empêcher ou réduire au minimum ces inconforts.

Notification de nouvelles informations

Il est possible que, pendant le déroulement de l'étude clinique, de nouvelles informations importantes sur HepaStem, le médicament étudié, deviennent disponibles. Vous serez informé(e) au moment opportun de tout élément nouveau susceptible de modifier votre décision de poursuivre votre participation à cette étude.

Si, au vu de ces nouvelles informations, vous décidez d'interrompre votre participation à l'étude, votre médecin investigateur veillera à ce que vous continuiez à recevoir le meilleur traitement possible.

Contraception, grossesse et allaitement

Participant de sexe féminin : Étant donné que les effets d'HepaStem sur un enfant à naître ou un nourrisson ne sont pas parfaitement connus, vous ne serez pas autorisée à participer à cette étude clinique si vous êtes enceinte, si vous souhaitez tomber enceinte ou si vous allaitez.

Si vous choisissez de participer à cette étude, vous devrez utiliser l'une des méthodes contraceptives autorisées (de manière à ne pas tomber enceinte). Votre médecin discutera avec vous des différentes options adéquates.

Bénéfices

Si vous acceptez de participer à cette étude, HepaStem pourra ou non s'avérer bénéfique pour le traitement de la maladie dont vous êtes atteint(e) ou soulager vos symptômes, en particulier pour aider à résoudre la décompensation aiguë actuelle de la fonction de votre foie.

Les informations obtenues grâce à cette étude peuvent contribuer à une meilleure connaissance de l'utilisation de ce médicament ou au développement d'un nouveau médicament pour le traitement de l'insuffisance hépatique aiguë sur maladie chronique du foie chez de futurs patients.

Traitement alternatif :

À ce jour, aucun traitement de votre affection approuvé par les autorités n'est disponible en Europe.

Le seul traitement actuellement disponible est la greffe de foie. Cependant, ce traitement est loin d'être idéal vu le manque d'organes pour la transplantation.

Retrait de l'étude

Votre participation est volontaire et vous avez le droit de vous retirer de l'étude à tout moment et sans devoir vous justifier. Néanmoins, il peut être utile pour le médecin investigateur et pour le promoteur de l'étude de savoir si vous vous retirez de l'étude car les contraintes liées au traitement sont trop importantes (par exemple, trop d'effets secondaires désagréables).

Il est aussi possible que le médecin investigateur vous retire de l'étude car vous êtes enceinte, car il/elle pense que c'est mieux pour votre santé ou car il/elle constate que vous ne respectez pas les consignes données aux participants.

Enfin, il est également possible que les autorités compétentes nationales ou internationales, le comité d'éthique qui a initialement approuvé l'étude ou le promoteur, décident d'interrompre l'étude.

Si vous retirez votre consentement à participer à l'étude, afin de garantir la validité de la recherche, les données encodées jusqu'au moment du retrait, seront conservées. Aucune nouvelle donnée ne sera envoyée au promoteur.

Si vous retirez votre consentement à participer à l'étude, vous pouvez contacter l'investigateur et demander que vos échantillons qui n'ont pas encore été utilisés, soient détruits. Les résultats obtenus de vos échantillons avant le retrait de votre consentement, restent la propriété du promoteur de l'étude.

Traitement après l'arrêt de l'étude

Dans toutes ces situations de retrait de l'étude mais également lorsque la période de participation prévue est arrivée à son terme, votre médecin investigateur évaluera votre état de santé et vous prescrira le meilleur traitement disponible.

Échantillons de matériel biologique collectés au cours de l'étude

Le promoteur de l'étude s'engage à ce que les échantillons (p. ex. sang, urine, tissu hépatique) ne soient utilisés que dans le cadre de l'étude.

- La procédure de codage des échantillons est la même que celle utilisée pour vos données médicales. Les échantillons envoyés au promoteur ne comprendront donc que votre code ID de l'étude.

- Le gestionnaire de ces échantillons (laboratoire d'hématologie des Cliniques St Luc, Bruxelles et Translational Research Center, KU Leuven, Louvain) s'engagent à les utiliser dans le contexte de la recherche clinique et de les détruire à la fin de la période de stockage prévue.

- L'échantillon de matière biologique prélevé est considéré comme un « don » et vous devez savoir que, en principe, vous ne recevrez aucun avantage financier (royalties) associé au développement d'un nouveau traitement dérivé de l'utilisation de votre don de matière biologique, qui pourrait présenter une valeur commerciale.

Le surplus de vos échantillons sera détruit dès que les analyses décrites dans ce document auront été réalisées (au plus tard, un an après la fin de l'étude).

Confidentialité et protection des données

Votre participation à l'étude signifie que vous consentez à la collecte de données à votre sujet par l'investigateur et à l'utilisation de ces données par le promoteur à des fins de recherches et en lien avec des publications scientifiques et médicales.
Vous avez le droit de demander à l'investigateur quelles données sont collectées à votre sujet et quelle est leur utilisation dans le cadre de l'étude. Ces données concernent votre état clinique actuel, mais également une partie de vos antécédents, les résultats des examens réalisés dans le contexte de votre

prise en charge selon les standards actuels et évidemment, les résultats des examens requis par le protocole. Vous avez le droit de consulter ces données et de les corriger si elles sont incorrectes. Ces droits sont garantis par la loi du 8 décembre 1992 relative à la protection de la vie privée à l'égard du traitement de données à caractère personnel et par la loi du 22 août 2002 relative aux droits du patient.
L'investigateur a un devoir de confidentialité vis-à-vis des données collectées.
Cela signifie qu'il/elle s'engage, non seulement à ne jamais révéler votre nom dans le contexte d'une publication ou d'une conférence, mais également qu'il/elle codera (votre identité sera remplacée par un code ID dans l'étude) vos données avant de les envoyer au gestionnaire de la banque des données collectées (Clinical Department, Promethera Biosciences).
L'investigateur et son équipe seront donc les seules personnes capables d'établir un lien entre les données transmises au cours de l'étude et votre dossier médical. Pour la présente étude, la loi exige que ce lien avec votre dossier soit conservé pendant minimum 30 ans et maximum 50 ans, loi belge du 19 décembre 2008 relative à l'obtention de matériel biologique humain et les arrêtés royaux qui s'y rapportent.
Les données personnelles transmises ne contiendront aucune combinaison d'éléments qui pourraient permettre de vous identifier.
Pour le gestionnaire des données de l'étude désigné par le promoteur, les données transmises ne permettront pas de vous identifier. Ce dernier est responsable de la collecte des données recueillies par tous les investigateurs participant à l'étude, leur traitement et leur protection selon les exigences de la loi belge relative à la protection de la vie privée.
Afin de vérifier la qualité de l'étude, il est possible que vos dossiers médicaux soient examinés par des personnes soumises au secret professionnel et désignées par le comité d'éthique, le promoteur de l'étude ou un audit indépendant. Dans tous les cas, l'examen de votre dossier médical sera sous l'entière responsabilité de l'investigateur et sous la supervision de l'un de ses collaborateurs, qu'il/elle aura désigné.
Les données de l'étude (codées) pourront être envoyées à des autorités de règlementation belges ou autres, aux comités d'éthique pertinents, à d'autres médecins et/ou à des organisations travaillant en collaboration avec le promoteur.
Elles pourront également être envoyées vers d'autres sites du promoteur en Belgique et dans d'autres pays où les standards en termes de protection des données personnelles peuvent être différents ou moins rigoureux. Comme indiqué ci-dessus, les données transmises sont codées. Le promoteur s'engage donc au respect des contraintes de la directive européenne et de la législation belge sur la protection de la vie privée.
Votre consentement à participer à la présente étude implique par conséquent également, votre consentement à l'utilisation de vos données médicales codées pour les objets décrits dans le présent feuillet d'information et à leur transmission aux personnes et autorités mentionnées ci-dessus.
Le promoteur s'engage à n'utiliser les données collectées que dans le contexte de l'étude à laquelle vous participez.

Assurance

Toute participation à une étude clinique comprend des risques, aussi faibles soient-ils. Même si aucune erreur n'a été commise, le promoteur assume la responsabilité de tout dommage causé au participant (ou dans le cas d'un décès, ses ayants-droits) et lié directement ou indirectement à sa participation à l'étude. Le promoteur a souscrit une assurance couvrant cette responsabilité.
Il vous est donc demandé d'informer l'investigateur de tout nouveau problème de santé avant de consulter un autre médecin, de prendre un autre médicament ou de recevoir tout autre traitement médical. Si pour une raison quelconque, vous consultez un autre médecin pendant la présente étude clinique, vous devez l'informer que vous participez à une étude clinique/lui présenter votre carte de

participant à une étude clinique. Ceci peut être important pour établir un diagnostic et traiter vos plaintes.
Si l'investigateur pense d'un lien avec l'étude est possible (l'assurance ne couvre pas la progression naturelle de votre maladie ou les effets secondaires connus de votre traitement normal), il/elle en informera le promoteur de l'étude, qui initiera la procédure de déclaration à la compagnie d'assurance. Cette dernière nommera un expert – si elle le considère nécessaire – pour établir s'il existe un lien entre votre nouveau problème de santé et l'étude.
Dans le cas d'un désaccord avec l'investigateur ou l'expert désigné par la compagnie d'assurance, et également si vous en ressentez la nécessité, vous ou – en cas de décès - vos ayants-droits pouvez entamer une procédure à l'égard de l'assureur, directement en Belgique (nom de la compagnie d'assurance, numéro de la police, contact).
La loi assure que l'assureur peut être convoqué par le juge de l'endroit où l'événement donnant lieu au dommage s'est déroulé, ou devant le juge de votre domicile, ou devant le juge du siège social de l'assureur.

Si vous participez à cette étude clinique, nous vous demandons :

- De ne pas boire d'alcool pendant au moins les 5 semaines suivant le début de votre participation.
- De collaborer pleinement au bon déroulement de cette étude.
- De ne dissimuler aucune information relative à votre état de santé, aux médicaments que vous prenez ou aux symptômes que vous présentez.
- De ne participer à aucune autre étude clinique impliquant un traitement expérimental (médicament, dispositif médical ou procédure) pendant votre participation à cette étude.
- De porter en permanence sur vous la « carte d'urgence ». Cette précaution est indispensable pour garantir votre sécurité si vous deviez recevoir des soins d'urgence dans une institution qui ne vous connaît pas.

Contact

Si vous avez besoin d'informations supplémentaires, mais aussi en cas de problème ou d'inquiétude, vous pouvez contacter le médecin investigateur …… ou un membre de son équipe de recherche …… au numéro de téléphone suivant (xx / xxx-xx-yy) ……..

En cas d'urgence ou en-dehors des heures de consultation, vous pouvez contacter le médiateur…… au numéro de téléphone suivant ……..., en mentionnant que vous participer à une étude clinique. Votre dossier contiendra les informations sur cette étude clinique, qui seront utiles pour le médecin de garde.

ÉTUDE HEP101

Étude Clinique multicentrique de phase II visant à évaluer la sécurité et l'efficacité préliminaire de 2 doses d'HepaStem chez des patients souffrant d'insuffisance hépatique aiguë sur une maladie chronique du foie.

Consentement éclairé du patient

ID du patient de l'étude : ……..

Participant

Je déclare que j'ai été informé(e) sur la nature de l'étude, son but, sa durée, les éventuels bénéfices et risques et ce que l'on attend de moi. J'ai pris connaissance du document d'information. J'ai eu suffisamment de temps pour y réfléchir et en parler avec une personne de mon choix, p. ex. mon médecin généraliste (MG) ou un membre de ma famille.

J'ai eu l'occasion de poser au médecin investigateur toutes les questions qui me sont venues à l'esprit et j'ai obtenu une réponse satisfaisante à mes questions.

Je comprends que ma participation à cette étude est volontaire et que je suis libre de mettre fin à ma participation à cette étude sans que cela ne modifie mes relations avec l'équipe thérapeutique en charge de ma santé.

Je comprends qu'au cours de cette étude clinique, mes données personnelles (en particulier mon dossier médical) seront récoltées, conservées et analysées. L'utilisation des informations relatives à ma santé est conforme aux dispositions légales et nécessite un consentement éclairé et volontaire préalable à ma participation à cette étude. Sans avoir signé le formulaire de consentement, je ne peux pas participer à l'étude.
Les données personnelles sont codées. J'ai le droit de consulter/corriger ces données.

Le consentement à la collecte et au traitement de mes données personnelles, en particulier des informations relatives à ma santé, est irrévocable. J'ai déjà été informé(e) que je peux mettre un terme à ma participation à cette étude à tout moment. Dans le cas où je retire mon consentement, je consens à ce que les données collectées jusqu'à ce moment-là puissent être analysées de manière pseudonyme (codées)

J'accepte que mes données soient conservées après la fin de l'étude ou mon interruption de l'étude, conformément aux exigences nationales requises.

J'accepte que les données d'étude collectées en vue d'atteindre les objectifs de cette étude puissent être traitées ultérieurement, pour autant que ce traitement soit limité au cadre de la présente étude pour une meilleure connaissance de la maladie et de son traitement.

J'accepte que le promoteur conserve des échantillons de matériel biologique récoltés en cours de l'étude après la fin de l'étude clinique, à des fins de recherches ultérieures mais limitées au cadre de la présente étude.

J'accepte que mon MG ou d'autres spécialistes en charge de ma santé soient informés de ma participation à cette étude clinique.

J'ai reçu une copie du document d'information du participant et du formulaire de consentement éclairé.

Prénom du patient : ……Nom du patient : ……

Signature : …… Date : ……

Représentant légal

Je déclare que j'ai été invité(e) à prendre une décision sur la participation éventuelle à l'étude clinique de la personne que je représente, en tenant compte de ses intérêts et de sa probable volonté. Mon consentement s'applique à tous les points repris dans le formulaire de consentement du participant.

J'ai également été informé(e) que, dès que la situation clinique le permettra, la personne que je représente sera mise au courant de sa participation à une étude clinique et qu'elle sera libre à ce moment-là de poursuivre cette participation ou d'y mettre un terme, en signant ou refusant de signer ce formulaire de consentement.

J'ai reçu une copie du document d'information du participant et du formulaire de consentement éclairé.

Prénom du représentant légal : ……Nom du représentant légal :……

Signature : …… Date : ……

Témoin/interprète

J'ai été présent pendant l'entièreté du processus d'information du patient et je confirme que les informations relatives aux objectifs et procédures de l'étude ont été fournies de manière adéquate, que le participant (ou son représentant légal) a apparemment compris l'étude et que le consentement à participer à l'étude a été donné librement.

Prénom et nom du témoin/de l'interprète : ……..

Signature : …… Date : ……

Médecin investigateur

Je soussigné(e), ……., médecin investigateur, confirme avoir fourni verbalement les informations nécessaires sur l'étude et avoir fourni une copie du document d'information au participant.

Je confirme qu'aucune pression n'a été exercée pour persuader le patient d'accepter de participer à l'étude et que je suis prêt(e) à répondre à toutes les questions supplémentaires si cela s'avère nécessaire.

Je confirme travailler en accord avec les principes éthiques énoncés dans la dernière version de la « Déclaration d'Helsinki », des « Bonnes pratiques Cliniques » et de la loi nationale en vigueur relative aux expérimentations sur la personne humaine.

Prénom du médecin investigateur : ……Nom du médecin investigateur : ……

Signature : …… Date : ……

ÉTUDE HEP101

ETUDE CLINIQUE MULTICENTRIQUE DE PHASE II VISANT À ÉVALUER LA SÉCURITÉ ET L'EFFICACITÉ PRÉLIMINAIRE DE 2 DOSES D'HEPASTEM CHEZ DES PATIENTS SOUFFRANT D'INSUFFISANCE HÉPATIQUE AIGUË SUR UNE MALADIE CHRONIQUE DU FOIE.

Information rétrospective

En raison de votre situation clinique au moment de votre admission aux soins intensifs, vous étiez incapable de décider vous-même si vous souhaitez ou non participer à l'étude mentionnée ci-dessus.

Il est alors habituel d'utiliser un représentant légal (généralement un proche), à qui l'on demande de prendre une décision sur la participation éventuelle d'une personne à l'étude en tenant compte de ses intérêts et de sa probable volonté.

Vous devez savoir que le comité d'éthique a accepté l'application de la procédure d'urgence dans le cadre de cette étude le ……

Vous avez donc été inclus(e) dans cette étude sans votre consentement préalable, en raison de la situation critique dans laquelle vous étiez.

Lorsque le comité d'éthique approuve l'application de la procédure d'urgence, le médecin investigateur demande dès que possible le consentement de votre représentant légal puis votre consentement personnel dès que l'amélioration de votre situation clinique vous permet d'être correctement informé(e) sur les objectifs et procédures de l'étude et de donner votre consentement à ce sujet.

Votre représentant légal a accepté que vous participiez à cette étude le....., sachant que, dès que votre situation clinique le permettrait, vous seriez informé(e) de votre participation à une étude clinique et que vous seriez à ce moment-là libre de décider de poursuivre votre participation ou de l'interrompre.

Nous vous demandons à présent de confirmer votre souhait de poursuivre cette participation ou de l'interrompre, et nous vous invitons à lire le document vous fournissant des informations complètes sur les objectifs et procédures de l'étude mais aussi sur les risques et bénéfices possibles liés au médicament expérimental et sur vos droits en tant que participant à une étude clinique.

Veuillez lire attentivement ces quelques pages d'information et poser toutes les questions que vous souhaitez au médecin investigateur ou à son représentant. Ce document comprend 2 parties : l'information essentielle à votre prise de décision et votre formulaire de consentement écrit.

HEP101 STUDIE
MULTICENTRALE FASE II VEILIGHEIDSSTUDIE EN EFFECTIVITEIT-VOORSTUDIE VAN 2 DOSERINGSREGIMES VAN HEPASTEM BIJ PATIËNTEN MET ACUUT-OP-CHRONISCHE LEVERFALEN

Patiënteninformatie

Deze patiënteninformatie en dit toestemmingsformulier na informatie zijn bedoeld voor patiënten die lijden aan acuut-op-chronisch leverfalen of hun wettelijke vertegenwoordigers die worden uitgenodigd om aan een wetenschappelijk onderzoek met HepaStem deel te nemen

Studiesponsor: **Promethera Biosciences**
Adres: **Rue Granbonpré 11**
1435 Mont St Guibert
België

Voornaamste onderzoeker: _____
Adres _____
Telefoonnummer: _____

Introductie

U wordt uitgenodigd om deel te nemen aan een klinische studie met het oog op de evaluatie van een medisch onderzoeksproduct voor de behandeling van uw ziekte. Een medisch onderzoeksproduct is een medisch product dat nog steeds wordt bestudeerd om de effectiviteit, veiligheid en werking ervan te evalueren.

Ter attentie van de patiënt: het is mogelijk dat u op het ogenblik dat u in de studie werd opgenomen, niet volledig in staat was om zelf te beslissen of u al dan niet aan de studie zou deelnemen.

In dat geval is het gebruikelijk om een wettelijke vertegenwoordiger in te schakelen, die wordt gevraagd om een beslissing te nemen m.b.t. de deelname van de betrokken persoon aan de studie, met het belang van de betrokken persoon voor ogen en rekening houdend met wat hij of zij waarschijnlijk had gewenst. Uw vertegenwoordiger keurde uw deelname goed, in de wetenschap dat wanneer uw klinische situatie het zou toelaten, u over uw deelname zou worden ingelicht en dat het u dan vrij zou staan om met uw deelname door te gaan of deze te beëindigen.

We vragen nu of u wenst te bevestigen of u al dan niet verder wenst deel te nemen.

.**Ter attentie van de wettelijke vertegenwoordiger:** wegens zijn/haar klinische situatie, wordt de persoon die u vertegenwoordigt, voor het ogenblik niet in staat geacht te beslissen of hij/zij al dan niet aan deze studie zal deelnemen, in volle bewustzijn van de implicaties. U wordt daarom gevraagd om te beslissen of hij/zij aan deze klinische studie deelneemt, met de belangen van de persoon die u vertegenwoordigt voor ogen en rekening houdend met zijn /haar waarschijnlijke wensen.

In de tekst die volgt, zijn de zinnen zo geformuleerd, als richten ze zich direct tot de persoon die u vertegenwoordigt.

De Onderzoeker (of de studiearts is de arts die verantwoordelijk is voor deze wetenschappelijke studie in het ziekenhuis) en de Sponsor (bedrijf dat het wetenschappelijk onderzoek start en financiert) hopen dat dit medisch product helpt bij de behandeling van patiënten met dezelfde ziekte als de uwe. Er is echter geen waarborg dat uw deelname aan deze studie uw gezondheid ten goede komt.

Vooraleer u instemt om aan deze studie deel te nemen, vragen we u om rekening te houden met de implicaties op het vlak van organisatie, mogelijke risico's en voordelen, wat u in staat moet stellen om, volledig bewust van de implicaties, een beslissing te nemen. Dit is gekend als het geven van een "geïnformeerde toestemming".

Gelieve deze informatie nauwkeurig te lezen en leg alle vragen waarmee u zit voor aan de onderzoeker of zijn/haar vertegenwoordiger. Dit document bestaat uit 2 delen: de informatie die essentieel is voor uw beslissing en, het ingevulde en ondertekende toestemmingsformulier.

Vooraleer u beslist, moet u bewust zijn van het volgende:

- Deze klinische studie wordt uitgevoerd nadat ze door een ethisch comité (in Leuven) werd beoordeeld na overleg met de ethische comités van andere deelnemende centra.
- Uw deelname is vrijwillig en moet verder zonder enige dwang verlopen. Een ondertekening van een document waarin u uw toestemming te kennen geeft, is vereist. Zelfs nadat u dit document hebt ondertekend, kunt u uw deelname stopzetten door de onderzoeker hierover te informeren. Uw beslissing om niet deel te nemen zal geen impact hebben op de kwaliteit van de zorg of op uw relatie met de onderzoeker.
- De gegevens die in deze studie worden verzameld zijn vertrouwelijk en uw anonimiteit is altijd gewaarborgd, ook tijdens de publicatie van de resultaten.
- De sponsor heeft een polis getekend bij een verzekeringsmaatschappij, ingeval u enige schade zou oplopen in verband met uw deelname aan deze studie.

  Verzekeringsmaatschappij: QBE Syndicate

  Polisnummer: 16ME305419EA
- Uw deelname aan de studie is volledig gratis. U zult geen kosten aangerekend krijgen voor de onderzoeken die specifiek zijn voor deze studie. U zult echter ook niet betaald worden voor uw deelname aan deze studie.
- Uw onkosten voor uw vervoer naar het ziekenhuis (bv. de kosten voor uw auto, taxi, treinticket, enz.) zullen u vergoed worden met waardebonnen (1 waardebon/uitgevoerd onderzoeksbezoek na uw ontslag uit het ziekenhuis). Neem contact op met het studieteam voor praktische informatie.
- De sponsor zal het ziekenhuis/de onderzoeker vergoeden voor alle kosten voor bezoeken/consultaties, onderzoeken of behandelingen die specifiek zijn voor deze studie.
- U mag de onderzoeker contacteren of een lid van zijn/haar team, telkens wanneer u bijkomende informatie wenst.

Doelstellingen en beschrijving van het studieprotocol

Wij nodigen u uit om deel te nemen aan een klinische studie waarbij gebruik wordt gemaakt van menselijke levercellen (progenitorcellen genoemd) afkomstig van gedoneerde levers. Van deze cellen verwacht men dat ze een gecombineerd systemisch (in heel uw lichaam) en lokaal effect op de lever hebben. Men verwacht dat ze een immunomodulerende functie hebben, wat betekent dat ze de overdreven, ontstekingsreactie die bij uw ziekte werd vastgesteld helpen regelen en vooral een oplossing bieden voor het huidige acute event. De cellen die worden getest worden heterologe (van een andere persoon) progenitorcellen genoemd, afkomstig van de lever van een volwassen persoon (HHALPC) of HepaStem.

De cellen zijn stamcellen, die uit volwassen levers zijn geïsoleerd en ze zijn geen embryonale stamcellen. Ze worden op een specifieke wijze bewaard en zijn goedgekeurd door de gezondheidsinstanties om te worden gebruikt bij patiënten in het kader van een klinisch onderzoek.

Twaalf (12) patiënten zullen in Europa in deze studie worden opgenomen, waaronder ongeveer 6 in België. De onderzoeker zal aan patiënten voorstellen om deel te nemen, waarbij zoals bij u, een diagnose van levercirrose en acuut op chronisch leverfalen werd vastgesteld.

Het voornaamste doel van deze studie bestaat erin om de veiligheid en effectiviteit te beoordelen van twee (2) doseringen van HepaStem 4 maal toegediend in een periode van 14 dagen (om de 3 dagen 1 keer toegediend).

Deze studie is een open studie van 2 doseringsregimes. Dit betekent dat u ofwel

- 4 x een lage dosis cellen (250 miljoen cellen elk) in een periode van 14 dagen krijgt toegediend (dit betekent de toediening van 1 spuit van 50 ml tweemaal per week gedurende 2 weken) of
- 4 x een hoge dosis cellen (500 miljoen cellen in een periode van 14 dagen) (dit betekent de toediening van 2 spuiten van 50 ml tweemaal per week gedurende 2 weken)

In beide gevallen zullen u en uw onderzoeker op de hoogte zijn van de dosis die u zal worden toegediend.

Verloop van de studie

Na een behandeling van 2 weken met cellen en nog eens 2 weken tijdens de observatieperiode, zal u in de studie blijven tot 1 jaar voorbij is (follow-up periode). Binnen de eerste maand zal u 8 studiebeoordelingen krijgen, d.w.z. dagen met procedures en onderzoeken die specifiek aan de studie gekoppeld zijn. Tijdens de follow-up periode zal u 4 studiebeoordelingen krijgen.

De onderzoeken en procedures die in verband met de studie nodig zijn, worden hieronder beschreven. Enkele onderzoeken en procedures maken deel uit van de standaardzorg die in het ziekenhuis wordt verleend, terwijl anderen in het kader van de studie worden aangeboden.

Uw deelname aan de studie zal over 3 periodes verlopen:

1 Screeningsperiode: in die periode zal uw onderzoeker verifiëren of u in aanmerking komt om aan de studie deel te nemen en hij zal uw medische geschiedenis en de gegevens vergaren van enkele onderzoeken die werden gedaan, nadat u had toegestemd om aan de studie deel te nemen.

Er zullen ten hoogste 25 mL bloed (ongeveer 2 eetlepels bloed) worden afgenomen. Bloedstalen zijn reeds genomen in het kader van de zorgverlening in uw huidige klinische situatie; daarom zal geen bijkomende prik in uw arm worden gegeven om deze buisjes bloed te verzamelen.

Er zal ook een analyse van uw urine plaatsvinden.
De bloed- en urinestalen zullen ons toelaten om de parameters te analyseren die ons meer zullen zeggen over de staat van uw organen (lever, nieren, bloed), over uw virologische status (waaronder hepatitis en HIV) alsook uw immuniteit.
Indien het nog niet is gebeurd, zal een echografie worden genomen van uw lever en hart, X-stralen van uw borstkas en een electrocardiogram. Deze onderzoeken zijn niet-invasieve behandelingen en doen geen pijn.
Indien u tijdens uw opname in het ziekenhuis een leverbiopsie hebt gehad, zullen de resultaten van dit onderzoek worden verzameld.
U zal een “noodkaart” met informatie over deze studie ontvangen. Houd deze kaart steeds bij u. Indien u in het ziekenhuis wordt opgenomen, toon dan die kaart aan uw arts en informeer hem/haar dat u aan deze studie deelneemt.

Na de screeningsperiode, zal u, nadat aan alle criteria is voldaan om aan deze studie deel te nemen, in staat zijn om de actieve periode in de studie aan te vangen en HepaStem te krijgen.

2 Actieve periode: Tijdens deze periode zal u in de loop van 14 dagen 4 x HepaStem toegediend krijgen. Afhankelijk van de groep waartoe u behoort, zal u 250 of 500 miljoen cellen worden toegediend. Dit beantwoordt aan een volume van 50 of 100 mL vloeistof.

HepaStem zal intraveneus worden toegediend, wat betekent dat een naald in een kleine of grote ader zal worden geplaatst om het product toe te dienen. Voorafgaand aan de HepaStem infusie zal medicatie worden gegeven om slechte reacties van het lichaam op het product te voorkomen.

Tijdens het toedienen en in elk geval 2, 3 en 4 weken na de eerste toediening, zal u klinische onderzoeken ondergaan en zullen er bloedstalen worden genomen (max 15mL bloed, ongeveer 1 eetlepels bloed).
De bloedstalen zullen toelaten om informatie in te winnen over de status van uw organen (lever, nieren, bloed).
Er zal een echografie van uw hart worden gemaakt na de eerste toediening en een echografie van uw lever voor elke infusie, 2 en 4 weken na de eerste toediening.

3 Follow-up periode: tijdens deze periode, zullen we u vragen om 2 maanden, 3 maanden, 6 maanden en 12 maanden na de eerste celinfusie naar het ziekenhuis terug te komen. Bij deze bezoeken zal u een klinisch onderzoek ondergaan en een bloedstaal (max 15mL bloed, ongeveer 1 eetlepels bloed). De resultaten van deze onderzoeken zullen worden verzameld.
De bloedstalen zullen ons toelaten om de parameters te analyseren die ons informatie zullen schenken over de status van uw organen (lever, nieren, bloed).
Indien u tijdens die periode een levertransplantatie hebt, zal indien mogelijk, een staal van uw verwijderde lever worden verzameld.

Na de follow-up periode, zullen we u vragen of u wilt instemmen om deel uit te maken van ons registratiesysteem voor bijkomende en regelmatige follow-up.

Risico's en ongemakken

A: Bijwerkingen van het medicijn van deze studie

- Alle medicijnen hebben gekende of onvoorspelbare bijwerkingen. Ook wanneer een voorafgaande studie heeft aangetoond dat HepaStem goed werd verdragen, kunt u toch de volgende bijwerkingen ervaren: **op korte termijn:**
  - trombose
- Ademhalingsmoeilijkheden overgevoeligheidsreactie of een reactie op de infusie: dit treedt op wanneer het immuunsysteem van het lichaam overmatig reageert op iets als medicatie. Overgevoeligheidsreacties kunnen omvatten: huidirritatie, roodheid, jeuk, zwelling, vochtafscheiding, korstvorming, huiduitslag, erupties, hoesten of kortademigheid, schorre stem, hoofdpijn, verstopte of lopende neus, niezen, rode (bloeddoorlopen) ogen, maagpijn, misselijkheid, braken, diarree, vermoeidheid, keelpijn, duizeligheid. Deze reacties kunnen schadelijk, ongemakkelijk of in sommige gevallen dodelijk zijn (in geval van anafylaxie): **op middellange of lange termijn:**
  - verspreiding in verschillende organen waar cellen de ontwikkeling van tumoren in de hand kunnen werken, hoewel dergelijke events zelden i.v.m. celtherapie werden gerapporteerd, een

immuniteitsreactie aangezien HepaStem bestaat uit cellen van een andere persoon, wat eventueel tot afstoting van cellen kan leiden.

Andere risico's en ongemakken die voor het ogenblik niet bekend zijn, zouden zich kunnen voordoen. Het is daarom heel belangrijk dat elk nieuw gezondheidsprobleem vlug aan de onderzoeker wordt gerapporteerd, ongeacht of u al dan niet denkt dat het met de studie te maken heeft.

C: Risico's in verband met procedures die specifiek zijn voor deze studie

Er zijn ook risico's/ ongemakken, die verband houden met specifieke onderzoeken die in verband met deze studie zullen worden uitgevoerd:

- De intraveneuze toediening kan het volgende veroorzaken:
  - Pijn, zoals voor elke injectie.
  - Infectie. Elke opening in de huid houdt een risico van infectie in, hoewel intraveneuze injectie een aseptische procedure is.
  - Flebitis: ontsteking van een ader, die door infectie kan worden veroorzaakt.
  - Infiltratie: infiltratie doet zich voor wanneer een intraveneuze vloeistof of medicatie per toeval in het omgevende weefsel eerder dan in de ader terechtkomt.
  - Embolie: een bloedklonter of vaste massa, zoals ook een luchtbel, kan door een IV in de bloedsomloop terechtkomen en een bloedvat blokkeren. Evenwel, het is bijna onmogelijk om op een gevaarlijke manier lucht door een perifere IV te injecteren. Het risico is groter met een centrale IV.
- Het **afnemen van bloed,** noodzakelijk, voor een analyse kan (zelden) pijn veroorzaken, bloeden, blauwe plekken of infectie bij de plaats van de injectie tot gevolg hebben. Ook kunnen patiënten zich tijdens de procedure duizelig voelen of ze kunnen flauw vallen.

Het personeel dat bloed afneemt, zal de beste medische zorgen toedienen om ongemakken te voorkomen of ze tot een minimum te herleiden.

Bekendmaking van nieuwe informatie

Het kan gebeuren dat belangrijke nieuwe informatie bij het onderzoek m.b.t. HepaStem ter beschikking komt te staan. U zal op tijd worden geïnformeerd over elk nieuw element dat uw beslissing kan beïnvloeden om verder aan deze studie deel te nemen.

Indien u, in het licht van de nieuwe informatie, beslist om verder aan de studie deel te nemen, zal uw onderzoeker ervoor zorgen dat u verder de beste behandeling krijgt.

Contraceptie, zwangerschap en borstvoeding

Vrouwelijke deelnemer**:** omdat de gevolgen van HepaStem op een ongeboren kind of zuigeling niet echt gekend zijn, zal u niet worden toegelaten om aan deze klinische studie deel te nemen, wanneer u zwanger bent, wenst zwanger te worden of indien u borstvoeding geeft.

Indien u aan deze studie wenst deel te nemen, moet u één van de goedgekeurde contraceptiemethodes gebruiken (zodat u niet zwanger wordt). Uw arts zal de verschillende geschikte opties met u bespreken.

Baten

Indien u akkoord gaat om aan deze studie deel te nemen, kan HepaStem al dan niet blijken een gunstige werking te hebben bij de behandeling van uw ziekte of bij het verlichten van de symptomen, vooral bij het helpen oplossen van de huidige acute decompensatie van de leverfunctie.

De informatie die men dankzij deze studie krijgt, kan ertoe bijdragen om een betere kennis te krijgen van het gebruik van dit medisch product of van de ontwikkeling van een nieuw medisch product voor de behandeling van acuut-op chronisch leverfalen bij toekomstige patiënten.

Alternatieve behandeling:

Geen enkele door de overheden goedgekeurde behandeling van uw toestand, is voor het ogenblik in Europa beschikbaar.

De enige behandeling die nu beschikbaar is, is levertransplantatie. Evenwel, deze behandeling is ver van ideaal, wegens gebrek aan transplantatieorganen.

Terugtrekking uit de studie

Uw deelname is vrijwillig en u hebt het recht om u te allen tijde uit de studie terug te trekken, zonder dat u een reden opgeeft. Evenwel, het kan voor de onderzoeker en de sponsor van de studie nuttig zijn om te weten of u zich terugtrekt wegens de te grote last veroorzaakt door de behandeling (te veel onaangename bijwerkingen, bijvoorbeeld).

Het is ook mogelijk dat de onderzoeker u uit de studie haalt, omdat u zwanger bent, omdat hij/zij denkt dat het beter is voor uw gezondheid of omdat hij/zij er achter is gekomen dat u de instructies die aan de deelnemers werden gegeven niet opvolgt.

Tenslotte kunnen de bevoegde nationale of internationale overheden, het ethisch comité dat de studie eerst goedkeurde of de sponsor beslissen om de studie stop te zetten.

Als u uw toestemming om deel te nemen aan de studie intrekt, dan zullen, om de geldigheid van het onderzoek te garanderen, de gecodeerde gegevens tot het punt waarop u zich terugtrok, bewaard blijven. Er mogen geen nieuwe gegevens naar de sponsor verzonden worden.

Als u uw goedkeuring om deel te nemen aan de studie intrekt, kunt u contact opnemen met de onderzoeker en vragen om de stalen die nog niet gebruikt zijn, te laten vernietigen. De resultaten verkregen uit uw stalen voordat u uw goedkeuring introk, blijven eigendom van de studiesponsor.

Behandeling nadat de studie werd stopgezet

In al deze situaties van terugtrekking uit de studie, maar ook wanneer de geplande periode van uw deelname is beëindigd, zal uw onderzoeker de staat van uw gezondheid onderzoeken en de best beschikbare behandeling voorschrijven.

Stalen van biologisch materiaal dat gedurende de studie werd verzameld

De sponsor van deze studie verzekert dat de stalen (zoals bloed, urine, weefsel van de lever) alleen in het kader van de studie zullen worden gebruikt.

- De procedure voor het coderen van stalen is dezelfde als deze die wordt gebruikt voor uw medische gegevens. Stalen die naar de sponsor worden verstuurd, zullen daarom alleen uw studie-ID-code bevatten.
- De beheerder van deze stalen (het hematologielaboratorium van de Cliniques St. Luc in Brussel en het Translational Research Center, KU Leuven, Leuven) zal ze gebruiken binnen de context van klinisch onderzoek en ze vernietigen aan het eind van de geplande bewaarperiode.
- Het staal van afgenomen biologisch materiaal wordt beschouwd als een ‘donatie’ en u moet er zich van bewust zijn dat u, in principe, geen enkel financieel voordeel (royalty’s) zult ontvangen geassocieerd met de ontwikkeling van nieuwe therapieën die zijn afgeleid van het gebruik van uw donatie van biologisch materiaal en die mogelijk commerciële waarde kunnen hebben.

Het overschot van stalen zal worden vernietigd zodra de analyses die in dit document zijn beschreven, werden uitgevoerd (ten laatste, één jaar na beëindiging van de studie)

Vertrouwelijkheid en gegevensbescherming

Uw deelname aan de studie betekent dat u ermee akkoord gaat dat de onderzoeker gegevens over u verzamelt en dat de studiesponsor deze gegevens gebruikt voor onderzoeksdoeleinden en in verband met wetenschappelijke en medische publicaties.

U hebt het recht om de onderzoeker te vragen welke gegevens er over u verzameld worden en waarvoor ze in functie van de studie gebruikt worden. Deze gegevens omvatten uw huidige klinische situatie, maar ook wat achtergrondgegevens, de resultaten van onderzoeken uitgevoerd binnen de context van uw gezondheidszorg in overeenstemming met de huidige standaarden en natuurlijk ook de resultaten van onderzoeken vereist door het protocol. U hebt het recht om deze gegevens te inspecteren en verbeteren als ze fout zouden zijn. Deze rechten zijn gegarandeerd door de wet van 8 december 1992 op de bescherming van de persoonlijke levenssfeer in functie van het verwerken van persoonlijke gegevens en door de wet van 22 augustus 2002 over de rechten van de patiënt.

De onderzoeker is gebonden door het beroepsgeheim over wat de verzamelde gegevens betreft.

Dit betekent dat hij/zij verplicht is om nooit uw naam te vermelden in de context van een publicatie of conferentie en dat hij/zij ook uw gegevens zal coderen (uw identiteit zal vervangen worden door een ID-code in de studie) alvorens ze naar de beheerder van de database van verzamelde gegevens te versturen (Clinical Department, Promethera Biosciences).

De onderzoeker en zijn/haar team zullen daarom de enigen zijn die een link kunnen maken tussen de gegevens die tijdens de studie verzonden worden en uw medisch dossier. Voor deze studie vereist de wet dat deze link met uw dossier gedurende minstens 30 jaar en maximaal 50 jaar bewaard blijft, in overeenstemming met de Belgische wet van 19 december 2008 over het gebruik van menselijk biologisch materiaal en de van toepassing zijnde koninklijke besluiten. De persoonlijke gegevens die verstuurd worden, zullen geen combinatie van elementen bevatten waaruit u geïdentificeerd kunt worden.

Voor de beheerder van de studiegegevens die wordt aangeduid door de sponsor, zullen de verstuurde gegevens het niet mogelijk maken om u te identificeren. De beheerder is verantwoordelijk voor het verzamelen van de gegevens van alle onderzoekers die deelnemen aan deze studie en ze te verwerken en beschermen in overeenstemming met de vereisten van de Belgische wet op de bescherming van de privacy.

Om de kwaliteit van de studie te verifiëren, is het mogelijk dat uw medisch dossier onderzocht wordt door personen die gebonden zijn door het beroepsgeheim en aangewezen door het ethisch comité, de sponsor van de studie of een onafhankelijk controle-orgaan. In elk geval mag dit onderzoek van uw medisch dossier alleen plaatsvinden onder de verantwoordelijkheid van de onderzoeker en onder toezicht van één van de door hem/haar aangeduide medewerkers.

De (gecodeerde) studiegegevens zullen mogelijk verstuurd worden naar Belgische of andere regelgevende autoriteiten, de relevante ethische comités, andere artsen en/of organisaties die samenwerken met de sponsor.

Ze zullen mogelijk ook verstuurd worden naar andere vestigingen van de sponsor in België en in andere landen waar de standaarden voor het beschermen van persoonlijke gegevens mogelijk anders of minder streng zijn. Zoals hierboven uitgelegd, worden de verstuurde gegevens gecodeerd. De sponsor verbindt zich ertoe de beperkingen van de Europese Richtlijn en de Belgische wetgeving op de bescherming van de privacy te respecteren.

Uw toestemming om deel te nemen aan deze studie impliceert hierdoor uw toestemming voor het gebruik van uw gecodeerde medische gegevens voor het doel beschreven in dit informatieformulier en voor de overdracht ervan naar de hierboven vermelde personen en autoriteiten.

De sponsor verbindt zich ertoe om de gegevens alleen te gebruiken binnen de context van de studie waaraan u deelneemt.

Verzekering

Elke deelname aan een klinische studie houdt een risico in, hoe klein ook. Zelfs zonder fout aanvaardt de sponsor verantwoordelijkheid voor schade die aan de deelnemer wordt veroorzaakt (of in het geval van overlijden, zijn/haar rechthebbenden) en die rechtstreeks of onrechtstreeks verbonden is met zijn/haar deelname aan de studie. De sponsor heeft een verzekering afgesloten voor deze verantwoordelijkheid.

We vragen u daarom om elk nieuwe gezondheidsprobleem te melden aan de onderzoeker voordat u een andere arts raadpleegt, andere medicatie begint in te nemen of een andere medische behandeling ondergaat. Als u, om eender welke reden, een andere arts raadpleegt tijdens deze klinische studie, moet u hem/haar inlichten dat u deelneemt aan een klinische studie/uw deelnemerskaart van de klinische studie tonen. Dit kan belangrijk zijn voor het stellen van een diagnose en het behandelen van uw klachten.

Als de onderzoeker denkt dat een verband met de studie mogelijk is (de verzekering dekt het natuurlijke verloop van uw ziekte of de bekende bijwerkingen van uw normale behandeling niet), zal hij/zij de studiesponsor informeren, die de aangifteprocedure bij de verzekeringsmaatschappij zal opstarten. De laatste zal een deskundige aanstellen (als dit nodig wordt geacht) om te beoordelen of er een verband bestaat tussen uw nieuwe gezondheidsproblemen en de studie.

In geval van onenigheid met de onderzoeker of met de door de verzekeringsmaatschappij aangestelde deskundige en ook wanneer u dat nodig acht, kunt u, of kunnen uw nabestaanden in geval van overlijden, de verzekeraar rechtstreeks in België dagvaarden (naam van verzekeringsmaatschappij, polisnummer, contactpersoon).

De wet voorziet dat de verzekeraar voor de rechtbank moet verschijnen van de locatie waar het schadegeval optrad, voor de rechtbank van uw woonplaats, of voor de rechtbank van de hoofdzetel van de verzekeraar.

Indien u aan deze klinische studie deelneemt, verzoeken wij om:

- Ten minste 5 weken voor het begin van uw deelname geen alcohol te drinken.
- Volledig mee te werken aan een rimpelloos verloop van de studie.
- Geen enkele informatie in verband met uw gezondheidstoestand, de medicatie die u neemt of de symptomen die u ondervindt, te verbergen.
- Niet deel te nemen aan een andere klinische studie waarbij er sprake is van een behandeling in het kader van het onderzoek, of het nu gaat om een medisch hulpmiddel of een procedure, terwijl men aan deze studie deelneemt.
- De "noodkaart" steeds bij u te hebben. Dit is absoluut noodzakelijk voor uw veiligheid in geval u dringende zorg nodig hebt in een instelling die u niet kent.

Contact

Indien u verder informatie nodig hebt, maar ook indien u problemen of zorgen heeft, kunt u de onderzoeker ……of een lid van zijn/haar research team..... op het volgende telefoonnummer (xx / xxx-xx-yy).....contacteren.

In noodgeval of buiten de consultatie-uren, contacteer de ombudsman …… op het volgend telefoonnummer …….. en vermeld dat u aan een klinische studie

deelneemt. Uw dossier zal informatie bevatten die nuttig is voor de dienstdoende arts in verband met deze klinische studie.

HEP101 STUDIE

MULTICENTRALE FASE II VEILIGHEIDSSTUDIE EN EFFECTIVITEIT-VOORSTUDIE VAN 2 DOSERINGSREGIMES VAN HEPASTEM BIJ PATIËNTEN MET ACUUT-OP-CHRONISCH LEVERFALEN

Toestemming van de geïnformeerde patiënt

ID van de patiënt in de studie: ……..

Deelnemer

Ik verklaar dat ik ingelicht ben over de aard van de studie, haar doelstelling, duur, risico's en baten, alsook over wat van mij wordt verwacht. Ik heb kennis genomen van het informatiedocument en ik heb voldoende tijd gehad om erover na te denken en te bespreken met een persoon van mijn keuze, zoals mijn huisarts of een familielid.

Ik heb de gelegenheid gehad om aan de onderzoeker de vragen te stellen die bij me opkwamen en ik heb een bevredigend antwoord op mijn vragen gekregen.

Ik begrijp dat mijn deelname aan deze studie vrijwillig is en dat ik vrij ben om mijn deelname aan deze studie te beëindigen zonder dat mijn relatie met het therapeutisch team dat verantwoordelijk is voor mijn gezondheid, in het gedrang komt.

Ik ben er me van bewust dat voor deze klinische studie mijn persoonlijke gegevens, vooral mijn medische gegevens worden verzameld, opgeslagen en geanalyseerd. Het gebruik van de informatie in verband met mijn gezondheid stemt overeen met de wettelijke bepalingen en vereist, voorafgaand aan deze studie een vrijwillig gegeven geïnformeerde toestemming. Zonder daaropvolgende toestemming kan ik niet aan de studie deelnemen.

De persoonlijke gegevens zijn geëncrypteerd. Ik heb het recht mijn gegevens te bekijken/te verbeteren.

De toestemming om mijn persoonlijke gegevens te verzamelen en te verwerken, vooral informatie over mijn gezondheid, is onherroeppelijk. Ik ben reeds geïnformeerd dat ik mijn deelname aan dit onderzoek te allen tijde kan beëindigen. Ingeval van een dergelijke terugtrekking van mijn toestemming, stem ik ermee in dat mijn gegevens die tot hiertoe zijn verzameld, onder pseudoniem (geëncrypteerd) kunnen worden geanalyseerd.

Ik stem ermee in dat mijn gegevens na beëindiging van de studie of bij onderbreking zullen worden opgeslagen volgens de nationale vereisten.

Ik stem ermee in dat de gegevens van deze studie, verzameld voor de doelstellingen van deze studie, op een later datum worden verwerkt, op voorwaarde dat de verwerking uitsluitend verband houdt met het kader van de huidige studie voor een beter begrip van de ziekte en haar behandeling.

Ik stem ermee in dat de sponsor stalen behoudt van het biologisch materiaal dat tijdens de studie, op het einde van het klinisch onderzoek voor verdere onderzoeksdoeleinden werd verzameld, maar binnen het kader van de huidige studie.

Ik stem ermee in dat mijn huisarts of andere specialisten die verantwoordelijk zijn voor mijn gezondheid, worden geïnformeerd over mijn deelname aan deze klinische studie.

Ik heb een kopie ontvangen van de informatie voor de deelnemer en een geïnformeerd toestemmingsformulier.

Achternaam van de patiënt: ……Voornaam: ……

Handtekening: …… Datum: ……

Wettelijk vertegenwoordiger

Ik verklaar dat me werd gevraagd om een beslissing te nemen voor de persoon die ik vertegenwoordig of de betrokkene al dan niet aan de klinische studie zou deelnemen, hierbij rekening houdend met wat het best voor hem/haar is en met zijn/haar vermoedelijke wensen. Mijn toestemming geldt voor alle punten die in de toestemming van de deelnemer zijn opgelijst.

Ik werd geïnformeerd dat zodra de klinische situatie het toelaat, de persoon die ik vertegenwoordiger, op de hoogte zal worden gebracht van zijn/haar deelname aan een klinische studie en dat hij/zij van dan af vrij zal zijn om haar deelname voort te zetten of te beëindigen door het toestemmingsformulier te ondertekenen of weigeren te ondertekenen.

Ik heb een kopie ontvangen van de informatie voor de deelnemer en een geïnformeerd toestemmingsformulier.

Achternaam van de wettelijke vertegenwoordiger: ……Voornaam ……

Handtekening: …… Datum: ……

Getuige/Tolk

Ik was aanwezig tijdens het volledige proces van de patiënteninformatie en ik bevestig dat de informatie over de doelstellingen en de procedures van de studie naar behoren werd verleend, dat de deelnemer (of zijn/haar vertegenwoordiger) blijkbaar de studie begreep en dat de toestemming om deel te nemen in volle vrijheid werd gegeven.

De getuige/tolk: … Achternaam & Voornaam: ……..

Handtekening: …… Datum : ……

De onderzoeker

Ik, de ondergetekende,……., onderzoeker, bevestig dat ik reeds mondeling de nodige informatie over de studie heb verstrekt en dat ik aan de deelnemer een kopie van het informatief document heb gegeven.

Ik bevestig dat er geen druk werd uitgeoefend om de patiënt te overtuigen om zijn toestemming te geven om aan deze studie deel te nemen en dat ik, zo nodig, bijkomende vragen wil beantwoorden.

Ik bevestig dat ik in overeenstemming met ethische principes handel die zijn opgenomen in de laatste versie van de “Verklaring van Helsinki”, de “Goede Klinische Praktijk” en de huidige nationale regeling, in verband met onderzoeken waaraan mensen deelnemen.

Achternaam van de onderzoeker: ……Voornaam……

Handtekening: …… Datum: ……

HEP101 STUDIE

MULTICENTRALE, FASE II VEILIGHEIDSSTUDIE EN EFFECTIVITEITS-VOORSTUDIE VAN 2 DOSERINGSREGIMES VAN HEPASTEM BIJ PATIËNTEN MET ACUUT –OP-CHRONISCH LEVERFALEN

Retrospectieve Informatie

Wegens uw klinische toestand toen u in intensieve zorgen werd opgenomen, was u niet in staat om zelf te beslissen of u al dan niet aan de hoger genoemde studie zou deelnemen.

In dat geval is het gebruikelijk om een wettelijke vertegenwoordiger in te schakelen (gewoonlijk dichte familie), aan wie wordt gevraagd om een beslissing te nemen in verband met de deelname van de betrokken persoon aan de studie in het belang van deze persoon en rekening houdend met zijn/haar waarschijnlijke wensen.

U dient er bewust van te zijn dat het ethisch comité instemde met de toepassing van de noodprocedure van deze studie op ……

U werd daarom in deze studie opgenomen zonder voorafgaande toestemming wegens de kritieke situatie waarin u zich bevond.

Wanneer het ethisch comité instemt met de noodprocedure, zal de onderzoeker zo vlug mogelijk de toestemming van de wettelijke vertegenwoordiger proberen te krijgen en uw toestemming, van zodra de verbetering van uw klinische situatie toelaat om juist te worden geïnformeerd over de doelstellingen en procedures van de studie en hiermee in te stemmen.

Uw vertegenwoordiger stemde in met uw deelname aan deze studie .....in de wetenschap dat u over uw deelname aan een klinische studie zou worden op de hoogte gebracht en dan vrij zou zijn om met verdere deelname in te stemmen of u uit de studie terug te trekken.

Wij vragen u nu te bevestigen of u al dan niet wenst deel te nemen en kennis te nemen van het document dat u over de doelstellingen en procedures van de studie volledig inlicht en ook over de mogelijke risico's en baten van de behandeling in het kader van dit onderzoek en uw rechten als een deelnemer aan de klinische studie.

Gelieve aandachtig deze paar bladzijden te lezen en stel alle vragen die u wenst te stellen aan de onderzoeker of zijn/haar vertegenwoordiger. Dit document is tweedelig: de informatie die voor uw beslissing van essentieel belang is en uw schriftelijke toestemming.

ÉTUDE HEP101

ÉTUDE CLINIQUE MULTICENTRIQUE DE PHASE II VISANT A EVALUER LA SECURITE ET L'EFFICACITE PRELIMINAIRE DE 2 DOSES D'HEPASTEM CHEZ DES PATIENTS SOUFFRANT D'INSUFFISANCE HEPATIQUE AIGUË SUR UNE MALADIE CHRONIQUE DU FOIE.

Formulaire d'information pour le patient

Ce formulaire d'information pour le patient et le formulaire de consentement éclairé sont destinés aux patients souffrant d'une insuffisance hépatique aiguë sur maladie chronique du foie (ACLF) qui sont invités à participer à une étude de recherche clinique réalisée avec HepaStem

Promoteur de l'étude : **Promethera Biosciences**
Adresse : **Rue Granbonpré 11**
1435 Mont St Guibert
Belgique

Investigateur principal : _____
Adresse : _____
Numéro de téléphone : _____

Introduction

Vous êtes invité(e) à participer à une étude clinique destinée à évaluer un médicament expérimental pour le traitement de votre maladie. Un médicament expérimental est un médicament faisant encore l'objet d'études pour évaluer son efficacité, sa sécurité d'emploi ou son mécanisme d'action.

L'investigateur (ou médecin de l'étude, c.-à-d. le médecin responsable de cette étude de recherche à l'hôpital) et le promoteur (la firme qui initie et finance cette recherche) espèrent que ce médicament expérimental peut présenter des avantages pour le traitement de patients atteints de la même maladie que la vôtre. Néanmoins, rien ne garantit que vous tiriez un bénéfice de votre participation à cette étude.

Avant que vous n'acceptiez de participer à cette étude, nous vous invitons à prendre connaissance de ses implications en termes d'organisation, risques et bénéfices éventuels, afin que vous puissiez prendre une décision en toute connaissance de cause. C'est ce qu'on appelle donner un « consentement éclairé ».

Veuillez lire attentivement ces quelques pages d'information et poser toutes les questions que vous souhaitez à l'investigateur ou à la personne qui le représente. Ce document comprend 2 parties : l'information essentielle à votre prise de décision et votre formulaire de consentement écrit.

Avant de prendre une décision, vous devez savoir que :

- Cette étude clinique est mise en œuvre après avoir fait l'objet d'une évaluation par un comité d'éthique (de Louvain) après consultation des comités d'éthique d'autres centres participants.
- Votre participation est volontaire et doit rester libre de toute contrainte. Elle nécessite la signature d'un document exprimant votre consentement. Même après avoir signé ce document, vous pouvez interrompre votre participation en informant le médecin investigateur. Votre décision de ne pas ou de ne plus participer à l'étude n'aura aucun impact sur la qualité de vos soins ni sur vos relations avec le médecin investigateur.

- Les données recueillies au cours de l'étude sont confidentielles. Votre anonymat sera toujours garanti, y compris lors de la publication des résultats.
- Le promoteur a souscrit une assurance au cas où vous subiriez un dommage lié à votre participation à cette étude clinique.

  Compagnie d'assurances : QBE Syndicate
  Numéro de la police : 16ME305419EA
- Votre participation à cette étude est totalement gratuite. Aucun frais ne vous sera facturé pour les examens spécifiques liés à cette étude. Cependant, vous ne serez pas rémunéré(e) pour votre participation à cette étude.
- Les dépenses liées à votre transport jusqu'à l'hôpital (comme les frais de voiture, de taxi, le ticket de train, etc.) vous seront remboursées avec des vouchers (1 pièce/visite de l'étude effectuée après votre sortie de l'hôpital). Veuillez contacter le personnel de l'étude pour les détails pratiques.
- Le promoteur remboursera l'hôpital/médecin investigateur pour tous les frais de visites/consultations, examens et traitements spécifiques à l'étude.
- Vous pouvez contacter le médecin investigateur ou un membre de son équipe à tout moment si vous avez besoin d'informations complémentaires.

Objectifs et description du protocole de l'étude

Vous êtes invité(e) à participer à une étude clinique portant sur le traitement par des cellules de foie humain (appelées « cellules progénitrices ») provenant de foies de donneurs. On espère que ces cellules ont un effet combiné systémique (dans tout le corps) et local dans le foie. Nous pensons qu'elles jouent un rôle immunomodulateur, c.-à-d. qu'elles aident à réguler la réponse inflammatoire exagérée qui est observée au cours de votre maladie, en particulier qu'elles aident à résoudre l'épisode aigu actuel de votre affection. Les cellules testées sont appelées « cellules progénitrices allogéniques (c.-à-d. provenant d'une autre personne) isolées à partir de foie humain adulte » ou HepaStem.
Les cellules sont des cellules souches isolées à partir de foies adultes et ne sont pas des cellules souches embryonnaires. Elles sont conservées selon une méthode spécifique et les autorités de santé ont approuvé leur utilisation chez des patients dans le cadre d'une étude clinique.

Douze (12) patients seront inclus dans cette étude en Europe, dont environ 6 en Belgique. Le médecin investigateur proposera à des patients qui, comme vous, présentent un diagnostic de cirrhose et une insuffisance hépatique aiguë sur maladie chronique du foie, de participer à cette étude.

L'objectif principal de cette étude est d'évaluer la sécurité et l'efficacité de deux (2) posologies d'HepaStem administrées 4 fois sur une période de 14 jours (1 administration tous les 3 jours).

Il s'agit d'une étude réalisée en ouvert pour évaluer 2 schémas posologiques, ce qui signifie que vous recevrez

- 4 administrations de la dose faible de cellules (250 millions de cellules pour chaque administration) sur une période de 14 jours (cela signifie l'administration d'1 seringue de 50 ml deux fois par semaine pendant 2 semaines), ou
- 4 administrations de la dose élevée de cellules (500 millions de cellules pour chaque administration) sur une période de 14 jours (cela signifie l'administration de 2 seringues de 50 ml deux fois par semaine pendant 2 semaines).

Dans les deux cas, vous et votre médecin investigateur saurez quelle posologie vous recevez.

Déroulement de l'étude

Après la phase de traitement de 2 semaines par les cellules et une période supplémentaire de 2 semaines de surveillance, vous resterez encore dans l'étude pendant 1 an (phase de suivi). Au cours du premier mois, vous ferez l'objet de 8 évaluations d'étude, c.-à-d. que vous subirez ces jours-là des procédures et des examens spécifiques à l'étude. Pendant la phase de suivi, vous ferez l'objet de 4 évaluations d'étude.

Les examens et procédures nécessaires liés à l'étude sont décrits ci-dessous. Certains de ces examens et procédures font partie des soins standards prodigués par votre hôpital, tandis que d'autres sont offerts dans le cadre de cette étude.

Votre participation à l'étude sera constituée de 3 phases :

1 Phase de sélection : pendant cette phase, votre médecin investigateur vérifiera si vous êtes éligible pour participer à cette étude, et établira un relevé de vos antécédents médicaux et des résultats de certains examens réalisés après avoir reçu votre accord pour participer à l'étude.

Tout au plus 25 ml supplémentaires de sang (environ 2 cuillères à soupe) seront prélevés. Des prises de sang sont déjà réalisées dans le cadre des soins liés à votre situation clinique actuelle ; aucune piqûre supplémentaire ne sera donc réalisée sur votre bras pour prélever ces tubes de sang.
Certaines analyses d'urine seront également réalisées.
Les échantillons de sang et d'urine permettront d'analyser des paramètres qui fourniront des informations sur l'état de vos organes (foie, reins, sang), sur votre statut virologique (notamment pour l'hépatite et l'infection à VIH) et sur votre immunité.
Si ce n'est pas déjà fait, on réalisera une échographie de votre foie et de votre cœur, une radiographie du thorax et un électrocardiogramme. Ces examens ne sont pas invasifs et ils sont indolores.
Si vous avez déjà subi une biopsie du foie pendant cette hospitalisation, les résultats de cet examen seront collectés.
Vous recevrez une « carte d'urgence » mentionnant toutes les informations sur l'étude. Veuillez porter cette carte sur vous en permanence. Si vous n'êtes pas hospitalisé(e), veuillez montrer cette carte à votre médecin et l'informer que vous participez à cette étude.

Après la phase de sélection, si vous répondez à tous les critères nécessaires pour entrer dans l'étude, vous serez en mesure de débuter la phase active de l'étude et vous recevrez le médicament HepaStem.

2 Phase active de l'étude : Pendant cette phase, vous recevrez 4 administrations d'HepaStem sur une période de 14 jours. En fonction du groupe auquel vous appartenez, vous recevrez 250 ou 500 millions de cellules par administration, ce qui équivaut à un volume de 50 ou 100 ml de liquide.

HepaStem sera administré par voie intraveineuse, ce qui signifie qu'une aiguille sera introduite dans une veine de petit ou grand calibre pour administrer le médicament. En vue de prévenir les réactions indésirables de votre corps au médicament, un médicament sera administré avant la perfusion d'HepaStem.

Pendant chaque administration et dans tous les cas, 2, 3 et 4 semaines après la 1ère administration, vous ferez l'objet d'examens cliniques et des échantillons de sang seront

prélevés (environ 15 mL de sang soit 1 cuillère à soupe). Les échantillons de sang permettront d'obtenir des informations sur l'état de vos organes (foie, reins, sang).
Une échographie de votre cœur sera réalisée après la 1ère administration et une échographie de votre foie sera réalisée après avant chaque perfusion, puis 2 et 4 semaines après la 1ère administration.

3_ Phase de suivi : pendant cette période, vous serez invité(e) à revenir à l'hôpital 2 mois, 3 mois, 6 mois et 12 mois après l'administration de la 1ère perfusion de cellules. Au cours de ces visites, vous ferez l'objet d'un examen clinique et un échantillon de sang sera prélevé (environ 15 mL de sang soit 1 cuillère à soupe). Les résultats de ces examens seront collectés.
Les échantillons de sang permettront d'analyser des paramètres qui fourniront des informations sur l'état de vos organes (foie, reins, sang).
Pendant cette période, si vous avez subi une transplantation du foie, un échantillon de votre foie explanté sera collecté si possible.

Après la phase de suivi, nous vous demanderons si vous acceptez d'être inscrit(e) dans notre registre dans le cadre d'un suivi régulier et complémentaire.

Risques et inconvénients

A : Effets secondaires du médicament de l'étude

- Tous les médicaments ont des effets secondaires connus ou imprévisibles. Même si l'étude précédente a révélé qu'HepaStem était bien toléré, il est toujours possible que vous présentiez les effets secondaires suivants : **à court terme :**
  - Thrombose
- Affection respiratoire Réaction d'hypersensibilité ou réaction à la perfusion : ceci se produit lorsque le système immunitaire du corps sur-réagit à quelque chose comme un médicament. La réaction d'hypersensibilité peut comprendre : irritation de la peau, rougeur, démangeaisons, gonflement, suintement, formation de croûtes, éruptions, toux ou essoufflement, enrouement de la voix, maux de tête, nez bouché ou qui coule, éternuements, yeux rouges, douleurs à l'estomac, nausée, vomissements, diarrhée, fatigue, mal de gorge, vertiges. Ces réactions peuvent être préjudiciables, inconfortables ou occasionnellement, fatales (en cas d'anaphylaxie). **à moyen ou long terme :**
  - distribution dans différents organes où les cellules peuvent favoriser le développement d'une tumeur, même si ces effets ont été rarement rapportés avec la thérapie cellulaire réaction immunitaire car HepaStem est constitué de cellules provenant d'une autre personne, ce qui peut éventuellement induire une réaction de rejet des cellules.

D'autres risques et inconvénients inconnus à ce jour pourraient également apparaitre. Il est donc très important de signaler rapidement tout nouveau problème de santé au médecin investigateur, que vous pensiez ou non qu'il soit en rapport avec l'étude.

C : Risques associés aux procédures spécifiques à l'étude

Il existe également des risques/inconvénients associés aux examens spécifiques qui seront réalisés dans le cadre de cette étude :

- L'administration intraveineuse peut provoquer les effets suivants :
  - Douleur, comme pour toute injection.

- Infection. Toute ouverture au niveau de la peau peut comporter un risque d'infection, même si l'insertion intraveineuse est une procédure aseptique.
- Phlébite : inflammation d'une veine pouvant être causée par une infection.
- Infiltration : une infiltration survient lorsqu'un liquide ou un médicament administré par voie intraveineuse pénètre accidentellement dans le tissu environnant plutôt que dans la veine.
- Embolie : un caillot sanguin ou une autre particule solide, ou encore une bulle d'air, peut être introduit dans la circulation au cours de l'administration IV et bloquer finalement un vaisseau. Cependant, il est presque impossible d'injecter de l'air au cours d'une administration IV par voie périphérique. Le risque est plus élevé en cas d'administration IV par voie centrale.

- La **prise de sang** nécessaire aux analyses peut (rarement) causer une douleur, un saignement, une contusion (bleu) ou une infection autour du site d'injection. Chez certains patients, des étourdissements, ou même un évanouissement, peuvent survenir pendant la procédure.

L'équipe qui réalisera la prise de sang fera usage des meilleurs soins médicaux pour empêcher ou réduire au minimum ces inconforts.

Notification de nouvelles informations

Il est possible que, pendant le déroulement de l'étude clinique, de nouvelles informations importantes sur HepaStem, le médicament étudié, deviennent disponibles. Vous serez informé(e) au moment opportun de tout élément nouveau susceptible de modifier votre décision de poursuivre votre participation à cette étude.

Si, au vu de ces nouvelles informations, vous décidez d'interrompre votre participation à l'étude, votre médecin investigateur veillera à ce que vous continuiez à recevoir le meilleur traitement possible.

Contraception, grossesse et allaitement

Participant de sexe féminin : Étant donné que les effets d'HepaStem sur un enfant à naître ou un nourrisson ne sont pas parfaitement connus, vous ne serez pas autorisée à participer à cette étude clinique si vous êtes enceinte, si vous souhaitez tomber enceinte ou si vous allaitez.

Si vous choisissez de participer à cette étude, vous devrez utiliser l'une des méthodes contraceptives autorisées (de manière à ne pas tomber enceinte). Votre médecin discutera avec vous des différentes options adéquates.

Bénéfices

Si vous acceptez de participer à cette étude, HepaStem pourra ou non s'avérer bénéfique pour le traitement de la maladie dont vous êtes atteint(e) ou soulager vos symptômes, en particulier pour aider à résoudre la décompensation aiguë actuelle de la fonction de votre foie.

Les informations obtenues grâce à cette étude peuvent contribuer à une meilleure connaissance de l'utilisation de ce médicament ou au développement d'un nouveau médicament pour le traitement de l'insuffisance hépatique aiguë sur maladie chronique du foie chez de futurs patients.

Traitement alternatif :

À ce jour, aucun traitement de votre affection approuvé par les autorités n'est disponible en Europe.

Le seul traitement actuellement disponible est la greffe de foie. Cependant, ce traitement est loin d'être idéal vu le manque d'organes pour la transplantation.

Retrait de l'étude

Votre participation est volontaire et vous avez le droit de vous retirer de l'étude à tout moment et sans devoir vous justifier. Néanmoins, il peut être utile pour le médecin investigateur et pour le promoteur

de l'étude de savoir si vous vous retirez de l'étude car les contraintes liées au traitement sont trop importantes (par exemple, trop d'effets secondaires désagréables).

Il est aussi possible que le médecin investigateur vous retire de l'étude car vous êtes enceinte, car il/elle pense que c'est mieux pour votre santé ou car il/elle constate que vous ne respectez pas les consignes données aux participants.

Enfin, il est également possible que les autorités compétentes nationales ou internationales, le comité d'éthique qui a initialement approuvé l'étude ou le promoteur, décident d'interrompre l'étude.

Si vous retirez votre consentement à participer à l'étude, afin de garantir la validité de la recherche, les données encodées jusqu'au moment du retrait, seront conservées. Aucune nouvelle donnée ne sera envoyée au promoteur.

Si vous retirez votre consentement à participer à l'étude, vous pouvez contacter l'investigateur et demander que vos échantillons qui n'ont pas encore été utilisés, soient détruits. Les résultats obtenus de vos échantillons avant le retrait de votre consentement, restent la propriété du promoteur de l'étude.

Traitement après l'arrêt de l'étude

Dans toutes ces situations de retrait de l'étude mais également lorsque la période de participation prévue est arrivée à son terme, votre médecin investigateur évaluera votre état de santé et vous prescrira le meilleur traitement disponible.

Échantillons de matériel biologique collectés au cours de l'étude

Le promoteur de l'étude s'engage à ce que les échantillons (p. ex. sang, urine, tissu hépatique) ne soient utilisés que dans le cadre de l'étude.

- La procédure de codage des échantillons est la même que celle utilisée pour vos données médicales. Les échantillons envoyés au promoteur ne comprendront donc que votre code ID de l'étude.

- Le gestionnaire de ces échantillons (laboratoire d'hématologie des Cliniques St Luc, Bruxelles et Translational Research Center, KU Leuven, Louvain) s'engagent à les utiliser dans le contexte de la recherche clinique et de les détruire à la fin de la période de stockage prévue.

- L'échantillon de matière biologique prélevé est considéré comme un « don » et vous devez savoir que, en principe, vous ne recevrez aucun avantage financier (royalties) associé au développement d'un nouveau traitement dérivé de l'utilisation de votre don de matière biologique, qui pourrait présenter une valeur commerciale.

Le surplus de vos échantillons sera détruit dès que les analyses décrites dans ce document auront été réalisées (au plus tard, un an après la fin de l'étude).

Confidentialité et protection des données

Votre participation à l'étude signifie que vous consentez à la collecte de données à votre sujet par l'investigateur et à l'utilisation de ces données par le promoteur à des fins de recherches et en lien avec des publications scientifiques et médicales.
Vous avez le droit de demander à l'investigateur quelles données sont collectées à votre sujet et quelle est leur utilisation dans le cadre de l'étude. Ces données concernent votre état clinique actuel, mais également une partie de vos antécédents, les résultats des examens réalisés dans le contexte de votre prise en charge selon les standards actuels et évidemment, les résultats des examens requis par le protocole. Vous avez le droit de consulter ces données et de les corriger si elles sont incorrectes. Ces droits sont garantis par la loi du 8 décembre 1992 relative à la protection de la vie privée à l'égard du traitement de données à caractère personnel et par la loi du 22 août 2002 relative aux droits du patient.
L'investigateur a un devoir de confidentialité vis-à-vis des données collectées.

Cela signifie qu'il/elle s'engage, non seulement à ne jamais révéler votre nom dans le contexte d'une publication ou d'une conférence, mais également qu'il/elle codera (votre identité sera remplacée par un code ID dans l'étude) vos données avant de les envoyer au gestionnaire de la banque des données collectées (Clinical Department, Promethera Biosciences).
L'investigateur et son équipe seront donc les seules personnes capables d'établir un lien entre les données transmises au cours de l'étude et votre dossier médical. Pour la présente étude, la loi exige que ce lien avec votre dossier soit conservé pendant minimum 30 ans et maximum 50 ans, loi belge du 19 décembre 2008 relative à l'obtention de matériel biologique humain et les arrêtés royaux qui s'y rapportent.
Les données personnelles transmises ne contiendront aucune combinaison d'éléments qui pourraient permettre de vous identifier.
Pour le gestionnaire des données de l'étude désigné par le promoteur, les données transmises ne permettront pas de vous identifier. Ce dernier est responsable de la collecte des données recueillies par tous les investigateurs participant à l'étude, leur traitement et leur protection selon les exigences de la loi belge relative à la protection de la vie privée.
Afin de vérifier la qualité de l'étude, il est possible que vos dossiers médicaux soient examinés par des personnes soumises au secret professionnel et désignées par le comité d'éthique, le promoteur de l'étude ou un audit indépendant. Dans tous les cas, l'examen de votre dossier médical sera sous l'entière responsabilité de l'investigateur et sous la supervision de l'un de ses collaborateurs, qu'il/elle aura désigné.
Les données de l'étude (codées) pourront être envoyées à des autorités de règlementation belges ou autres, aux comités d'éthique pertinents, à d'autres médecins et/ou à des organisations travaillant en collaboration avec le promoteur.
Elles pourront également être envoyées vers d'autres sites du promoteur en Belgique et dans d'autres pays où les standards en termes de protection des données personnelles peuvent être différents ou moins rigoureux. Comme indiqué ci-dessus, les données transmises sont codées. Le promoteur s'engage donc au respect des contraintes de la directive européenne et de la législation belge sur la protection de la vie privée.
Votre consentement à participer à la présente étude implique par conséquent également, votre consentement à l'utilisation de vos données médicales codées pour les objets décrits dans le présent feuillet d'information et à leur transmission aux personnes et autorités mentionnées ci-dessus.
Le promoteur s'engage à n'utiliser les données collectées que dans le contexte de l'étude à laquelle vous participez.

Assurance

Toute participation à une étude clinique comprend des risques, aussi faibles soient-ils. Même si aucune erreur n'a été commise, le promoteur assume la responsabilité de tout dommage causé au participant (ou dans le cas d'un décès, ses ayants-droits) et lié directement ou indirectement à sa participation à l'étude. Le promoteur a souscrit une assurance couvrant cette responsabilité.
Il vous est donc demandé d'informer l'investigateur de tout nouveau problème de santé avant de consulter un autre médecin, de prendre un autre médicament ou de recevoir tout autre traitement médical. Si pour une raison quelconque, vous consultez un autre médecin pendant la présente étude clinique, vous devez l'informer que vous participez à une étude clinique/lui présenter votre carte de participant à une étude clinique. Ceci peut être important pour établir un diagnostic et traiter vos plaintes.
Si l'investigateur pense d'un lien avec l'étude est possible (l'assurance ne couvre pas la progression naturelle de votre maladie ou les effets secondaires connus de votre traitement normal), il/elle en informera le promoteur de l'étude, qui initiera la procédure de déclaration à la compagnie

d'assurance. Cette dernière nommera un expert – si elle le considère nécessaire – pour établir s'il existe un lien entre votre nouveau problème de santé et l'étude.
Dans le cas d'un désaccord avec l'investigateur ou l'expert désigné par la compagnie d'assurance, et également si vous en ressentez la nécessité, vous ou – en cas de décès - vos ayants-droits pouvez entamer une procédure à l'égard de l'assureur, directement en Belgique (nom de la compagnie d'assurance, numéro de la police, contact).
La loi assure que l'assureur peut être convoqué par le juge de l'endroit où l'événement donnant lieu au dommage s'est déroulé, ou devant le juge de votre domicile, ou devant le juge du siège social de l'assureur.

Si vous participez à cette étude clinique, nous vous demandons :

- De ne pas boire d'alcool pendant au moins les 5 semaines suivant le début de votre participation.
- De collaborer pleinement au bon déroulement de cette étude.
- De ne dissimuler aucune information relative à votre état de santé, aux médicaments que vous prenez ou aux symptômes que vous présentez.
- De ne participer à aucune autre étude clinique impliquant un traitement expérimental (médicament, dispositif médical ou procédure) pendant votre participation à cette étude.
- De porter en permanence sur vous la « carte d'urgence ». Cette précaution est indispensable pour garantir votre sécurité si vous deviez recevoir des soins d'urgence dans une institution qui ne vous connaît pas.

Contact

Si vous avez besoin d'informations supplémentaires, mais aussi en cas de problème ou d'inquiétude, vous pouvez contacter le médecin investigateur …… ou un membre de son équipe de recherche ……. au numéro de téléphone suivant (xx / xxx-xx-yy) ……..

En cas d'urgence ou en-dehors des heures de consultation, vous pouvez contacter le service des urgences …… au numéro de téléphone suivant ……..., en mentionnant que vous participer à une étude clinique. Votre dossier contiendra les informations sur cette étude clinique, qui seront utiles pour le médecin de garde.

ÉTUDE HEP101

Étude Clinique multicentrique de phase II visant à évaluer la sécurité et l'efficacité préliminaire de 2 doses d'HepaStem chez des patients souffrant d'insuffisance hépatique aiguë sur une maladie chronique du foie.

Consentement éclairé du patient

ID du patient de l'étude : ……..

Participant

Je déclare que j'ai été informé(e) sur la nature de l'étude, son but, sa durée, les éventuels bénéfices et risques et ce que l'on attend de moi. J'ai pris connaissance du document d'information. J'ai eu suffisamment de temps pour y réfléchir et en parler avec une personne de mon choix, p. ex. mon médecin généraliste (MG) ou un membre de ma famille.

J'ai eu l'occasion de poser au médecin investigateur toutes les questions qui me sont venues à l'esprit et j'ai obtenu une réponse satisfaisante à mes questions.

Je comprends que ma participation à cette étude est volontaire et que je suis libre de mettre fin à ma participation à cette étude sans que cela ne modifie mes relations avec l'équipe thérapeutique en charge de ma santé.

Je comprends qu'au cours de cette étude clinique, mes données personnelles (en particulier mon dossier médical) seront récoltées, conservées et analysées. L'utilisation des informations relatives à ma santé est conforme aux dispositions légales et nécessite un consentement éclairé et volontaire préalable à ma participation à cette étude. Sans avoir signé le formulaire de consentement, je ne peux pas participer à l'étude.
Les données personnelles sont codées. J'ai le droit de consulter/corriger ces données.

Le consentement à la collecte et au traitement de mes données personnelles, en particulier des informations relatives à ma santé, est irrévocable. J'ai déjà été informé(e) que je peux mettre un terme à ma participation à cette étude à tout moment. Dans le cas où je retire mon consentement, je consens à ce que les données collectées jusqu'à ce moment-là puissent être analysées de manière pseudonyme (codées)

J'accepte que mes données soient conservées après la fin de l'étude ou mon interruption de l'étude, conformément aux exigences nationales requises.

J'accepte que les données d'étude collectées en vue d'atteindre les objectifs de cette étude puissent être traitées ultérieurement, pour autant que ce traitement soit limité au cadre de la présente étude pour une meilleure connaissance de la maladie et de son traitement.

J'accepte que le promoteur conserve des échantillons de matériel biologique récoltés en cours de l'étude après la fin de l'étude clinique, à des fins de recherches ultérieures mais limitées au cadre de la présente étude.

J'accepte que mon MG ou d'autres spécialistes en charge de ma santé soient informés de ma participation à cette étude clinique.

J'ai reçu une copie du document d'information du participant et du formulaire de consentement éclairé.

Prénom du patient : ……Nom du patient : ……

Signature : …… Date : ……

Témoin/interprète

J'ai été présent pendant l'entièreté du processus d'information du patient et je confirme que les informations relatives aux objectifs et procédures de l'étude ont été fournies de manière adéquate, que le participant (ou son représentant légal) a apparemment compris l'étude et que le consentement à participer à l'étude a été donné librement.

Prénom et nom du témoin/de l'interprète : ……..

Signature : …… Date : ……

Médecin investigateur

Je soussigné(e), ……., médecin investigateur, confirme avoir fourni verbalement les informations nécessaires sur l'étude et avoir fourni une copie du document d'information au participant.

Je confirme qu'aucune pression n'a été exercée pour persuader le patient d'accepter de participer à l'étude et que je suis prêt(e) à répondre à toutes les questions supplémentaires si cela s'avère nécessaire.

Je confirme travailler en accord avec les principes éthiques énoncés dans la dernière version de la « Déclaration d'Helsinki », des « Bonnes pratiques Cliniques » et de la loi nationale en vigueur relative aux expérimentations sur la personne humaine.

Prénom du médecin investigateur : ……Nom du médecin investigateur : ……

Signature : …… Date : ……

HEP101 STUDIE
MULTICENTRALE FASE II VEILIGHEIDSSTUDIE EN EFFECTIVITEIT-VOORSTUDIE VAN 2 DOSERINGSREGIMES VAN HEPASTEM BIJ PATIËNTEN MET ACUUT-OP-CHRONISCHE LEVERFALEN

Patiënteninformatie

Deze patiënteninformatie en dit toestemmingsformulier na informatie zijn bedoeld voor patiënten die lijden aan acuut-op-chronisch leverfalen die worden uitgenodigd om aan een wetenschappelijk onderzoek met HepaStem deel te nemen

Studiesponsor: **Promethera Biosciences**
Adres: **Rue Granbonpré 11**
1435 Mont St Guibert
België

Voornaamste onderzoeker: _____
Adres _____
Telefoonnummer: _____

Introductie

U wordt uitgenodigd om deel te nemen aan een klinische studie met het oog op de evaluatie van een medisch onderzoeksproduct voor de behandeling van uw ziekte. Een medisch onderzoeksproduct is een medisch product dat nog steeds wordt bestudeerd om de effectiviteit, veiligheid en werking ervan te evalueren.

De Onderzoeker (of de studiearts is de arts die verantwoordelijk is voor deze wetenschappelijke studie in het ziekenhuis) en de Sponsor (bedrijf dat het wetenschappelijk onderzoek start en financiert) hopen dat dit medisch product helpt bij de behandeling van patiënten met dezelfde ziekte als de uwe. Er is echter geen waarborg dat uw deelname aan deze studie uw gezondheid ten goede komt.

Vooraleer u instemt om aan deze studie deel te nemen, vragen we u om rekening te houden met de implicaties op het vlak van organisatie, mogelijke risico's en voordelen, wat u in staat moet stellen om, volledig bewust van de implicaties, een beslissing te nemen. Dit is gekend als het geven van een "geïnformeerde toestemming".

Gelieve deze informatie nauwkeurig te lezen en leg alle vragen waarmee u zit voor aan de onderzoeker of zijn/haar vertegenwoordiger. Dit document bestaat uit 2 delen: de informatie die essentieel is voor uw beslissing en, het ingevulde en ondertekende toestemmingsformulier.

Vooraleer u beslist, moet u bewust zijn van het volgende:

- Deze klinische studie wordt uitgevoerd nadat ze door een ethisch comité (in Leuven) werd beoordeeld na overleg met de ethische comités van andere deelnemende centra.
- Uw deelname is vrijwillig en moet verder zonder enige dwang verlopen. Een ondertekening van een document waarin u uw toestemming te kennen geeft, is vereist. Zelfs nadat u dit document hebt ondertekend, kunt u uw deelname stopzetten door de onderzoeker hierover te

informeren. Uw beslissing om niet deel te nemen zal geen impact hebben op de kwaliteit van de zorg of op uw relatie met de onderzoeker.

- De gegevens die in deze studie worden verzameld zijn vertrouwelijk en uw anonimiteit is altijd gewaarborgd, ook tijdens de publicatie van de resultaten.
- De sponsor heeft een polis getekend bij een verzekeringsmaatschappij, ingeval u enige schade zou oplopen in verband met uw deelname aan deze studie.

  Verzekeringsmaatschappij: QBE Syndicate

  Polisnummer: 16ME305419EA
- Uw deelname aan de studie is volledig gratis. U zult geen kosten aangerekend krijgen voor de onderzoeken die specifiek zijn voor deze studie. U zult echter ook niet betaald worden voor uw deelname aan deze studie.
- Uw onkosten voor uw vervoer naar het ziekenhuis (bv. de kosten voor uw auto, taxi, treinticket, enz.) zullen u vergoed worden met waardebonnen (1 waardebon/uitgevoerd onderzoeksbezoek na uw ontslag uit het ziekenhuis). Neem contact op met het studieteam voor praktische informatie.
- De sponsor zal het ziekenhuis/de onderzoeker vergoeden voor alle kosten voor bezoeken/consultaties, onderzoeken of behandelingen die specifiek zijn voor deze studie.
- U mag de onderzoeker contacteren of een lid van zijn/haar team, telkens wanneer u bijkomende informatie wenst.

Doelstellingen en beschrijving van het studieprotocol

Wij nodigen u uit om deel te nemen aan een klinische studie waarbij gebruik wordt gemaakt van menselijke levercellen (progenitorcellen genoemd) afkomstig van gedoneerde levers. Van deze cellen verwacht men dat ze een gecombineerd systemisch (in heel uw lichaam) en lokaal effect op de lever hebben. Men verwacht dat ze een immunomodulerende functie hebben, wat betekent dat ze de overdreven, ontstekingsreactie die bij uw ziekte werd vastgesteld helpen regelen en vooral een oplossing bieden voor het huidige acute event. De cellen die worden getest worden heterologe (van een andere persoon) progenitorcellen genoemd, afkomstig van de lever van een volwassen persoon (HHALPC) of HepaStem.

De cellen zijn stamcellen, die uit volwassen levers zijn geïsoleerd en ze zijn geen embryonale stamcellen. Ze worden op een specifieke wijze bewaard en zijn goedgekeurd door de gezondheidsinstanties om te worden gebruikt bij patiënten in het kader van een klinisch onderzoek.

Twaalf (12) patiënten zullen in Europa in deze studie worden opgenomen, waaronder ongeveer 6 in België. De onderzoeker zal aan patiënten voorstellen om deel te nemen, waarbij zoals bij u, een diagnose van levercirrose en acuut op chronisch leverfalen werd vastgesteld.

Het voornaamste doel van deze studie bestaat erin om de veiligheid en effectiviteit te beoordelen van twee (2) doseringen van HepaStem 4 maal toegediend in een periode van 14 dagen (om de 3 dagen 1 keer toegediend).

Deze studie is een open studie van 2 doseringsregimes. Dit betekent dat u ofwel

- 4 x een lage dosis cellen (250 miljoen cellen elk) in een periode van 14 dagen krijgt toegediend (dit betekent de toediening van 1 spuit van 50 ml tweemaal per week gedurende 2 weken) of

- 4 x een hoge dosis cellen (500 miljoen cellen in een periode van 14 dagen) (dit betekent de toediening van 2 spuiten van 50 ml tweemaal per week gedurende 2 weken)

In beide gevallen zullen u en uw onderzoeker op de hoogte zijn van de dosis die u zal worden toegediend.

Verloop van de studie

Na een behandeling van 2 weken met cellen en nog eens 2 weken tijdens de observatieperiode, zal u in de studie blijven tot 1 jaar voorbij is (follow-up periode). Binnen de eerste maand zal u 8 studiebeoordelingen krijgen, d.w.z. dagen met procedures en onderzoeken die specifiek aan de studie gekoppeld zijn. Tijdens de follow-up periode zal u 4 studiebeoordelingen krijgen.

De onderzoeken en procedures die in verband met de studie nodig zijn, worden hieronder beschreven. Enkele onderzoeken en procedures maken deel uit van de standaardzorg die in het ziekenhuis wordt verleend, terwijl anderen in het kader van de studie worden aangeboden.

Uw deelname aan de studie zal over 3 periodes verlopen:

1_ Screeningsperiode: in die periode zal uw onderzoeker verifiëren of u in aanmerking komt om aan de studie deel te nemen en hij zal uw medische geschiedenis en de gegevens vergaren van enkele onderzoeken die werden gedaan, nadat u had toegestemd om aan de studie deel te nemen.

Er zullen ten hoogste 25 mL bloed (ongeveer 2 eetlepels bloed) worden afgenomen. Bloedstalen zijn reeds genomen in het kader van de zorgverlening in uw huidige klinische situatie; daarom zal geen bijkomende prik in uw arm worden gegeven om deze buisjes bloed te verzamelen.

Er zal ook een analyse van uw urine plaatsvinden.
De bloed- en urinestalen zullen ons toelaten om de parameters te analyseren die ons meer zullen zeggen over de staat van uw organen (lever, nieren, bloed), over uw virologische status (waaronder hepatitis en HIV) alsook uw immuniteit.
Indien het nog niet is gebeurd, zal een echografie worden genomen van uw lever en hart, X-stralen van uw borstkas en een electrocardiogram. Deze onderzoeken zijn niet-invasieve behandelingen en doen geen pijn.
Indien u tijdens uw opname in het ziekenhuis een leverbiopsie hebt gehad, zullen de resultaten van dit onderzoek worden verzameld.
U zal een “noodkaart” met informatie over deze studie ontvangen. Houd deze kaart steeds bij u. Indien u in het ziekenhuis wordt opgenomen, toon dan die kaart aan uw arts en informeer hem/haar dat u aan deze studie deelneemt.

Na de screeningsperiode, zal u, nadat aan alle criteria is voldaan om aan deze studie deel te nemen, in staat zijn om de actieve periode in de studie aan te vangen en HepaStem te krijgen.

2_ Actieve periode: Tijdens deze periode zal u in de loop van 14 dagen 4 x HepaStem toegediend krijgen. Afhankelijk van de groep waartoe u behoort, zal u 250 of 500 miljoen cellen worden toegediend. Dit beantwoordt aan een volume van 50 of 100 mL vloeistof.

HepaStem zal intraveneus worden toegediend, wat betekent dat een naald in een kleine of grote ader zal worden geplaatst om het product toe te dienen. Voorafgaand aan de HepaStem infusie zal medicatie worden gegeven om slechte reacties van het lichaam op het product te voorkomen.

Tijdens het toedienen en in elk geval 2, 3 en 4 weken na de eerste toediening, zal u klinische onderzoeken ondergaan en zullen er bloedstalen worden genomen (max 15mL bloed, ongeveer 1 eetlepels bloed).
De bloedstalen zullen toelaten om informatie in te winnen over de status van uw organen (lever, nieren, bloed).
Er zal een echografie van uw hart worden gemaakt na de eerste toediening en een echografie van uw lever voor elke infusie, 2 en 4 weken na de eerste toediening.

3_ Follow-up periode: tijdens deze periode, zullen we u vragen om 2 maanden, 3 maanden, 6 maanden en 12 maanden na de eerste celinfusie naar het ziekenhuis terug te komen. Bij deze bezoeken zal u een klinisch onderzoek ondergaan en een bloedstaal (max 15mL bloed, ongeveer 1 eetlepels bloed). De resultaten van deze onderzoeken zullen worden verzameld.
De bloedstalen zullen ons toelaten om de parameters te analyseren die ons informatie zullen schenken over de status van uw organen (lever, nieren, bloed).
Indien u tijdens die periode een levertransplantatie hebt, zal indien mogelijk, een staal van uw verwijderde lever worden verzameld.

Na de follow-up periode, zullen we u vragen of u wilt instemmen om deel uit te maken van ons registratiesysteem voor bijkomende en regelmatige follow-up.

Risico's en ongemakken

A: Bijwerkingen van het medicijn van deze studie

- Alle medicijnen hebben gekende of onvoorspelbare bijwerkingen. Ook wanneer een voorafgaande studie heeft aangetoond dat HepaStem goed werd verdragen, kunt u toch de volgende bijwerkingen ervaren: **op korte termijn:**
  - trombose
- Ademhalingsmoeilijkheden overgevoeligheidsreactie of een reactie op de infusie: dit treedt op wanneer het immuunsysteem van het lichaam overmatig reageert op iets als medicatie. Overgevoeligheidsreacties kunnen omvatten: huidirritatie, roodheid, jeuk, zwelling, vochtafscheiding, korstvorming, huiduitslag, erupties, hoesten of kortademigheid, schorre stem, hoofdpijn, verstopte of lopende neus, niezen, rode (bloeddoorlopen) ogen, maagpijn, misselijkheid, braken, diarree, vermoeidheid, keelpijn, duizeligheid. Deze reacties kunnen schadelijk, ongemakkelijk of in sommige gevallen dodelijk zijn (in geval van anafylaxie): **op middellange of lange termijn:**
  - verspreiding in verschillende organen waar cellen de ontwikkeling van tumoren in de hand kunnen werken, hoewel dergelijke events zelden i.v.m. celtherapie werden gerapporteerd, een immuniteitsreactie aangezien HepaStem bestaat uit cellen van een andere persoon, wat eventueel tot afstoting van cellen kan leiden.

Andere risico's en ongemakken die voor het ogenblik niet bekend zijn, zouden zich kunnen voordoen. Het is daarom heel belangrijk dat elk nieuw gezondheidsprobleem vlug aan de onderzoeker wordt gerapporteerd, ongeacht of u al dan niet denkt dat het met de studie te maken heeft.

C: Risico's in verband met procedures die specifiek zijn voor deze studie

Er zijn ook risico's/ ongemakken, die verband houden met specifieke onderzoeken die in verband met deze studie zullen worden uitgevoerd:

- De intraveneuze toediening kan het volgende veroorzaken:

  - Pijn, zoals voor elke injectie.
  - Infectie. Elke opening in de huid houdt een risico van infectie in, hoewel intraveneuze injectie een aseptische procedure is.
  - Flebitis: ontsteking van een ader, die door infectie kan worden veroorzaakt.
  - Infiltratie: infiltratie doet zich voor wanneer een intraveneuze vloeistof of medicatie per toeval in het omgevende weefsel eerder dan in de ader terechtkomt.
  - Embolie: een bloedklonter of vaste massa, zoals ook een luchtbel, kan door een IV in de bloedsomloop terechtkomen en een bloedvat blokkeren. Evenwel, het is bijna onmogelijk om op een gevaarlijke manier lucht door een perifere IV te injecteren. Het risico is groter met een centrale IV.
- Het **afnemen van bloed,** noodzakelijk, voor een analyse kan (zelden) pijn veroorzaken, bloeden, blauwe plekken of infectie bij de plaats van de injectie tot gevolg hebben. Ook kunnen patiënten zich tijdens de procedure duizelig voelen of ze kunnen flauw vallen.

Het personeel dat bloed afneemt, zal de beste medische zorgen toedienen om ongemakken te voorkomen of ze tot een minimum te herleiden.

Bekendmaking van nieuwe informatie

Het kan gebeuren dat belangrijke nieuwe informatie bij het onderzoek m.b.t. HepaStem ter beschikking komt te staan. U zal op tijd worden geïnformeerd over elk nieuw element dat uw beslissing kan beïnvloeden om verder aan deze studie deel te nemen.

Indien u, in het licht van de nieuwe informatie, beslist om verder aan de studie deel te nemen, zal uw onderzoeker ervoor zorgen dat u verder de beste behandeling krijgt.

Contraceptie, zwangerschap en borstvoeding

Vrouwelijke deelnemer**:** omdat de gevolgen van HepaStem op een ongeboren kind of zuigeling niet echt gekend zijn, zal u niet worden toegelaten om aan deze klinische studie deel te nemen, wanneer u zwanger bent, wenst zwanger te worden of indien u borstvoeding geeft.

Indien u aan deze studie wenst deel te nemen, moet u één van de goedgekeurde contraceptiemethodes gebruiken (zodat u niet zwanger wordt). Uw arts zal de verschillende geschikte opties met u bespreken.

Baten

Indien u akkoord gaat om aan deze studie deel te nemen, kan HepaStem al dan niet blijken een gunstige werking te hebben bij de behandeling van uw ziekte of bij het verlichten van de symptomen, vooral bij het helpen oplossen van de huidige acute decompensatie van de leverfunctie.

De informatie die men dankzij deze studie krijgt, kan ertoe bijdragen om een betere kennis te krijgen van het gebruik van dit medisch product of van de ontwikkeling van een nieuw medisch product voor de behandeling van acuut-op chronisch leverfalen bij toekomstige patiënten.

Alternatieve behandeling:

Geen enkele door de overheden goedgekeurde behandeling van uw toestand, is voor het ogenblik in Europa beschikbaar.

De enige behandeling die nu beschikbaar is, is levertransplantatie. Evenwel, deze behandeling is ver van ideaal, wegens gebrek aan transplantatieorganen.

Terugtrekking uit de studie

Uw deelname is vrijwillig en u hebt het recht om u te allen tijde uit de studie terug te trekken, zonder dat u een reden opgeeft. Evenwel, het kan voor de onderzoeker en de sponsor van de studie nuttig zijn om te weten of u zich terugtrekt wegens de te grote last veroorzaakt door de behandeling (te veel onaangename bijwerkingen, bijvoorbeeld).

Het is ook mogelijk dat de onderzoeker u uit de studie haalt, omdat u zwanger bent, omdat hij/zij denkt dat het beter is voor uw gezondheid of omdat hij/zij er achter is gekomen dat u de instructies die aan de deelnemers werden gegeven niet opvolgt.

Tenslotte kunnen de bevoegde nationale of internationale overheden, het ethisch comité dat de studie eerst goedkeurde of de sponsor beslissen om de studie stop te zetten.

Als u uw toestemming om deel te nemen aan de studie intrekt, dan zullen, om de geldigheid van het onderzoek te garanderen, de gecodeerde gegevens tot het punt waarop u zich terugtrok, bewaard blijven. Er mogen geen nieuwe gegevens naar de sponsor verzonden worden.

Als u uw goedkeuring om deel te nemen aan de studie intrekt, kunt u contact opnemen met de onderzoeker en vragen om de stalen die nog niet gebruikt zijn, te laten vernietigen. De resultaten verkregen uit uw stalen voordat u uw goedkeuring introk, blijven eigendom van de studiesponsor.

Behandeling nadat de studie werd stopgezet

In al deze situaties van terugtrekking uit de studie, maar ook wanneer de geplande periode van uw deelname is beëindigd, zal uw onderzoeker de staat van uw gezondheid onderzoeken en de best beschikbare behandeling voorschrijven.

Stalen van biologisch materiaal dat gedurende de studie werd verzameld

De sponsor van deze studie verzekert dat de stalen (zoals bloed, urine, weefsel van de lever) alleen in het kader van de studie zullen worden gebruikt.

- De procedure voor het coderen van stalen is dezelfde als deze die wordt gebruikt voor uw medische gegevens. Stalen die naar de sponsor worden verstuurd, zullen daarom alleen uw studie-ID-code bevatten.
- De beheerder van deze stalen (het hematologielaboratorium van de Cliniques St. Luc in Brussel en het Translational Research Center, KU Leuven, Leuven) zal ze gebruiken binnen de context van klinisch onderzoek en ze vernietigen aan het eind van de geplande bewaarperiode.
- Het staal van afgenomen biologisch materiaal wordt beschouwd als een ‘donatie’ en u moet er zich van bewust zijn dat u, in principe, geen enkel financieel voordeel (royalty’s) zult ontvangen geassocieerd met de ontwikkeling van nieuwe therapieën die zijn afgeleid van het gebruik van uw donatie van biologisch materiaal en die mogelijk commerciële waarde kunnen hebben.

Het overschot van stalen zal worden vernietigd zodra de analyses die in dit document zijn beschreven, werden uitgevoerd (ten laatste, één jaar na beëindiging van de studie)

Vertrouwelijkheid en gegevensbescherming

Uw deelname aan de studie betekent dat u ermee akkoord gaat dat de onderzoeker gegevens over u verzamelt en dat de studiesponsor deze gegevens gebruikt voor onderzoeksdoeleinden en in verband met wetenschappelijke en medische publicaties.

U hebt het recht om de onderzoeker te vragen welke gegevens er over u verzameld worden en waarvoor ze in functie van de studie gebruikt worden. Deze gegevens omvatten uw huidige klinische situatie, maar ook wat achtergrondgegevens, de resultaten van onderzoeken uitgevoerd binnen de

context van uw gezondheidszorg in overeenstemming met de huidige standaarden en natuurlijk ook de resultaten van onderzoeken vereist door het protocol. U hebt het recht om deze gegevens te inspecteren en verbeteren als ze fout zouden zijn. Deze rechten zijn gegarandeerd door de wet van 8 december 1992 op de bescherming van de persoonlijke levenssfeer in functie van het verwerken van persoonlijke gegevens en door de wet van 22 augustus 2002 over de rechten van de patiënt.

De onderzoeker is gebonden door het beroepsgeheim over wat de verzamelde gegevens betreft.

Dit betekent dat hij/zij verplicht is om nooit uw naam te vermelden in de context van een publicatie of conferentie en dat hij/zij ook uw gegevens zal coderen (uw identiteit zal vervangen worden door een ID-code in de studie) alvorens ze naar de beheerder van de database van verzamelde gegevens te versturen (Clinical Department, Promethera Biosciences).

De onderzoeker en zijn/haar team zullen daarom de enigen zijn die een link kunnen maken tussen de gegevens die tijdens de studie verzonden worden en uw medisch dossier. Voor deze studie vereist de wet dat deze link met uw dossier gedurende minstens 30 jaar en maximaal 50 jaar bewaard blijft, in overeenstemming met de Belgische wet van 19 december 2008 over het gebruik van menselijk biologisch materiaal en de van toepassing zijnde koninklijke besluiten. De persoonlijke gegevens die verstuurd worden, zullen geen combinatie van elementen bevatten waaruit u geïdentificeerd kunt worden.

Voor de beheerder van de studiegegevens die wordt aangeduid door de sponsor, zullen de verstuurde gegevens het niet mogelijk maken om u te identificeren. De beheerder is verantwoordelijk voor het verzamelen van de gegevens van alle onderzoekers die deelnemen aan deze studie en ze te verwerken en beschermen in overeenstemming met de vereisten van de Belgische wet op de bescherming van de privacy.

Om de kwaliteit van de studie te verifiëren, is het mogelijk dat uw medisch dossier onderzocht wordt door personen die gebonden zijn door het beroepsgeheim en aangewezen door het ethisch comité, de sponsor van de studie of een onafhankelijk controle-orgaan. In elk geval mag dit onderzoek van uw medisch dossier alleen plaatsvinden onder de verantwoordelijkheid van de onderzoeker en onder toezicht van één van de door hem/haar aangeduide medewerkers.

De (gecodeerde) studiegegevens zullen mogelijk verstuurd worden naar Belgische of andere regelgevende autoriteiten, de relevante ethische comités, andere artsen en/of organisaties die samenwerken met de sponsor.

Ze zullen mogelijk ook verstuurd worden naar andere vestigingen van de sponsor in België en in andere landen waar de standaarden voor het beschermen van persoonlijke gegevens mogelijk anders of minder streng zijn. Zoals hierboven uitgelegd, worden de verstuurde gegevens gecodeerd. De sponsor verbindt zich ertoe de beperkingen van de Europese Richtlijn en de Belgische wetgeving op de bescherming van de privacy te respecteren.

Uw toestemming om deel te nemen aan deze studie impliceert hierdoor uw toestemming voor het gebruik van uw gecodeerde medische gegevens voor het doel beschreven in dit informatieformulier en voor de overdracht ervan naar de hierboven vermelde personen en autoriteiten.

De sponsor verbindt zich ertoe om de gegevens alleen te gebruiken binnen de context van de studie waaraan u deelneemt.

Verzekering

Elke deelname aan een klinische studie houdt een risico in, hoe klein ook. Zelfs zonder fout aanvaardt de sponsor verantwoordelijkheid voor schade die aan de deelnemer wordt veroorzaakt (of in het geval van overlijden, zijn/haar rechthebbenden) en die rechtstreeks of onrechtstreeks

verbonden is met zijn/haar deelname aan de studie. De sponsor heeft een verzekering afgesloten voor deze verantwoordelijkheid.

We vragen u daarom om elk nieuwe gezondheidsprobleem te melden aan de onderzoeker voordat u een andere arts raadpleegt, andere medicatie begint in te nemen of een andere medische behandeling ondergaat. Als u, om eender welke reden, een andere arts raadpleegt tijdens deze klinische studie, moet u hem/haar inlichten dat u deelneemt aan een klinische studie/uw deelnemerskaart van de klinische studie tonen. Dit kan belangrijk zijn voor het stellen van een diagnose en het behandelen van uw klachten.

Als de onderzoeker denkt dat een verband met de studie mogelijk is (de verzekering dekt het natuurlijke verloop van uw ziekte of de bekende bijwerkingen van uw normale behandeling niet), zal hij/zij de studiesponsor informeren, die de aangifteprocedure bij de verzekeringsmaatschappij zal opstarten. De laatste zal een deskundige aanstellen (als dit nodig wordt geacht) om te beoordelen of er een verband bestaat tussen uw nieuwe gezondheidsproblemen en de studie.

In geval van onenigheid met de onderzoeker of met de door de verzekeringsmaatschappij aangestelde deskundige en ook wanneer u dat nodig acht, kunt u, of kunnen uw nabestaanden in geval van overlijden, de verzekeraar rechtstreeks in België dagvaarden (naam van verzekeringsmaatschappij, polisnummer, contactpersoon).

De wet voorziet dat de verzekeraar voor de rechtbank moet verschijnen van de locatie waar het schadegeval optrad, voor de rechtbank van uw woonplaats, of voor de rechtbank van de hoofdzetel van de verzekeraar.

Indien u aan deze klinische studie deelneemt, verzoeken wij om:

- Ten minste 5 weken na het begin van uw deelname geen alcohol te drinken.
- Volledig mee te werken aan een rimpelloos verloop van de studie.
- Geen enkele informatie in verband met uw gezondheidstoestand, de medicatie die u neemt of de symptomen die u ondervindt, te verbergen.
- Niet deel te nemen aan een andere klinische studie waarbij er sprake is van een behandeling in het kader van het onderzoek, of het nu gaat om een medisch hulpmiddel of een procedure, terwijl men aan deze studie deelneemt.
- De "noodkaart" steeds bij u te hebben. Dit is absoluut noodzakelijk voor uw veiligheid in geval u dringende zorg nodig hebt in een instelling die u niet kent.

Contact

Indien u verder informatie nodig hebt, maar ook indien u problemen of zorgen heeft, kunt u de onderzoeker ……of een lid van zijn/haar research team..... op het volgende telefoonnummer (xx / xxx-xx-yy).....contacteren.

In noodgeval of buiten de consultatie-uren, contacteer de spoedgevallen …… op het volgend telefoonnummer ……... en vermeld dat u aan een klinische studie deelneemt. Uw dossier zal informatie bevatten die nuttig is voor de dienstdoende arts in verband met deze klinische studie.

HEP101 STUDIE

MULTICENTRALE FASE II VEILIGHEIDSSTUDIE EN EFFECTIVITEIT-VOORSTUDIE VAN 2 DOSERINGSREGIMES VAN HEPASTEM BIJ PATIËNTEN MET ACUUT-OP-CHRONISCH LEVERFALEN

Toestemming van de geïnformeerde patiënt

ID van de patiënt in de studie: ……..

Deelnemer

Ik verklaar dat ik ingelicht ben over de aard van de studie, haar doelstelling, duur, risico's en baten, alsook over wat van mij wordt verwacht. Ik heb kennis genomen van het informatiedocument en ik heb voldoende tijd gehad om erover na te denken en te bespreken met een persoon van mijn keuze, zoals mijn huisarts of een familielid.

Ik heb de gelegenheid gehad om aan de onderzoeker de vragen te stellen die bij me opkwamen en ik heb een bevredigend antwoord op mijn vragen gekregen.

Ik begrijp dat mijn deelname aan deze studie vrijwillig is en dat ik vrij ben om mijn deelname aan deze studie te beëindigen zonder dat mijn relatie met het therapeutisch team dat verantwoordelijk is voor mijn gezondheid, in het gedrang komt.

Ik ben er me van bewust dat voor deze klinische studie mijn persoonlijke gegevens, vooral mijn medische gegevens worden verzameld, opgeslagen en geanalyseerd. Het gebruik van de informatie in verband met mijn gezondheid stemt overeen met de wettelijke bepalingen en vereist, voorafgaand aan deze studie een vrijwillig gegeven geïnformeerde toestemming. Zonder daaropvolgende toestemming kan ik niet aan de studie deelnemen.

De persoonlijke gegevens zijn geëncrypteerd. Ik heb het recht mijn gegevens te bekijken/te verbeteren.

De toestemming om mijn persoonlijke gegevens te verzamelen en te verwerken, vooral informatie over mijn gezondheid, is onherroeppelijk. Ik ben reeds geïnformeerd dat ik mijn deelname aan dit onderzoek te allen tijde kan beëindigen. Ingeval van een dergelijke terugtrekking van mijn toestemming, stem ik ermee in dat mijn gegevens die tot hiertoe zijn verzameld, onder pseudoniem (geëncrypteerd) kunnen worden geanalyseerd.

Ik stem ermee in dat mijn gegevens na beëindiging van de studie of bij onderbreking zullen worden opgeslagen volgens de nationale vereisten.

Ik stem ermee in dat de gegevens van deze studie, verzameld voor de doelstellingen van deze studie, op een later datum worden verwerkt, op voorwaarde dat de verwerking uitsluitend verband houdt met het kader van de huidige studie voor een beter begrip van de ziekte en haar behandeling.

Ik stem ermee in dat de sponsor stalen behoudt van het biologisch materiaal dat tijdens de studie, op het einde van het klinisch onderzoek voor verdere onderzoeksdoeleinden werd verzameld, maar binnen het kader van de huidige studie.

Ik stem ermee in dat mijn huisarts of andere specialisten die verantwoordelijk zijn voor mijn gezondheid, worden geïnformeerd over mijn deelname aan deze klinische studie.

Ik heb een kopie ontvangen van de informatie voor de deelnemer en een geïnformeerd toestemmingsformulier.

Achternaam van de patiënt: ……Voornaam: ……

Handtekening: …… Datum: ……

Getuige/Tolk

Ik was aanwezig tijdens het volledige proces van de patiënteninformatie en ik bevestig dat de informatie over de doelstellingen en de procedures van de studie naar behoren werd verleend, dat de deelnemer (of zijn/haar vertegenwoordiger) blijkbaar de studie begreep en dat de toestemming om deel te nemen in volle vrijheid werd gegeven.

De getuige/tolk: … Achternaam & Voornaam: ……..

Handtekening: …… Datum : ……

De onderzoeker

Ik, de ondergetekende,……., onderzoeker, bevestig dat ik reeds mondeling de nodige informatie over de studie heb verstrekt en dat ik aan de deelnemer een kopie van het informatief document heb gegeven.

Ik bevestig dat er geen druk werd uitgeoefend om de patiënt te overtuigen om zijn toestemming te geven om aan deze studie deel te nemen en dat ik, zo nodig, bijkomende vragen wil beantwoorden.

Ik bevestig dat ik in overeenstemming met ethische principes handel die zijn opgenomen in de laatste versie van de “Verklaring van Helsinki”, de “Goede Klinische Praktijk” en de huidige nationale regeling, in verband met onderzoeken waaraan mensen deelnemen.

Achternaam van de onderzoeker: ……Voornaam……

Handtekening: …… Datum: ……

ÉTUDE HEP101

ÉTUDE CLINIQUE MULTICENTRIQUE DE PHASE II VISANT A EVALUER LA SECURITE ET L'EFFICACITE PRELIMINAIRE DE 2 DOSES D'HEPASTEM CHEZ DES PATIENTS SOUFFRANT D'INSUFFISANCE HEPATIQUE AIGUË SUR UNE MALADIE CHRONIQUE DU FOIE.

Formulaire d'information pour le patient

Ce formulaire d'information pour le patient et le formulaire de consentement éclairé sont destinés aux patients souffrant d'une insuffisance hépatique aiguë sur maladie chronique du foie (ACLF) et aux représentants légaux de ces patients qui sont invités à participer à une étude de recherche clinique réalisée avec HepaStem

Promoteur de l'étude : **Promethera Biosciences**
Adresse : **Rue Granbonpré 11**
1435 Mont St Guibert
Belgique

Investigateur principal : _____
Adresse : _____
Numéro de téléphone : _____

Introduction

Vous êtes invité(e) à participer à une étude clinique destinée à évaluer un médicament expérimental pour le traitement de votre maladie. Un médicament expérimental est un médicament faisant encore l'objet d'études pour évaluer son efficacité, sa sécurité d'emploi ou son mécanisme d'action.

À l'attention du patient : Il peut arriver qu'au moment de votre inclusion dans l'étude, vous n'étiez pas pleinement capable de décider vous-même si vous souhaitiez participer on non à cette étude. Il est alors habituel d'utiliser un représentant légal, à qui l'on demande de confirmer votre décision sur votre participation à l'étude, en tenant compte de vos intérêts et de votre probable volonté.

À l'attention du représentant légal : En raison de sa situation clinique, on estime que la personne que vous représentez est capable de décider si elle souhaite ou non participer à cette étude, mais sa pleine conscience des implications liées à l'étude peut être remise en cause de par sa maladie. Vous êtes dès lors invité(e) à confirmer si la personne que vous représentez veut participer ou non à cette étude clinique, en tenant compte de ses intérêts et de sa probable volonté.

Dans la suite de ce document, le texte est rédigé comme s'il s'adressait directement à la personne que vous représentez.

L'investigateur (ou médecin de l'étude, c.-à-d. le médecin responsable de cette étude de recherche à l'hôpital) et le promoteur (la firme qui initie et finance cette recherche) espèrent que ce médicament expérimental peut présenter des avantages pour le traitement de patients atteints de la même maladie que la vôtre. Néanmoins, rien ne garantit que vous tiriez un bénéfice de votre participation à cette étude.

Avant que vous n'acceptiez de participer à cette étude, nous vous invitons à prendre connaissance de ses implications en termes d'organisation, risques et bénéfices éventuels, afin que vous puissiez prendre une décision en toute connaissance de cause. C'est ce qu'on appelle donner un « consentement éclairé ».

Veuillez lire attentivement ces quelques pages d'information et poser toutes les questions que vous souhaitez à l'investigateur ou à la personne qui le représente. Ce document comprend 2 parties : l'information essentielle à votre prise de décision et votre formulaire de consentement écrit.

Avant de prendre une décision, vous devez savoir que :

- Cette étude clinique est mise en œuvre après avoir fait l'objet d'une évaluation par un comité d'éthique (de Louvain) après consultation des comités d'éthique d'autres centres participants.
- Votre participation est volontaire et doit rester libre de toute contrainte. Elle nécessite la signature d'un document exprimant votre consentement. Même après avoir signé ce document, vous pouvez interrompre votre participation en informant le médecin investigateur. Votre décision de ne pas ou de ne plus participer à l'étude n'aura aucun impact sur la qualité de vos soins ni sur vos relations avec le médecin investigateur.
- Les données recueillies au cours de l'étude sont confidentielles. Votre anonymat sera toujours garanti, y compris lors de la publication des résultats.
- Le promoteur a souscrit une assurance au cas où vous subiriez un dommage lié à votre participation à cette étude clinique.

  Compagnie d'assurances : QBE Syndicate
  Numéro de la police : 16ME305419EA
- Votre participation à cette étude est totalement gratuite. Aucun frais ne vous sera facturé pour les examens spécifiques liés à cette étude. Cependant, vous ne serez pas rémunéré(e) pour votre participation à cette étude.
- Les dépenses liées à votre transport jusqu'à l'hôpital (comme les frais de voiture, de taxi, le ticket de train, etc.) vous seront remboursées avec des vouchers (1 pièce/visite de l'étude effectuée après votre sortie de l'hôpital). Veuillez contacter le personnel de l'étude pour les détails pratiques.
- Le promoteur remboursera l'hôpital/médecin investigateur pour tous les frais de visites/consultations, examens et traitements spécifiques à l'étude.
- Vous pouvez contacter le médecin investigateur ou un membre de son équipe à tout moment si vous avez besoin d'informations complémentaires.

Objectifs et description du protocole de l'étude

Vous êtes invité(e) à participer à une étude clinique portant sur le traitement par des cellules de foie humain (appelées « cellules progénitrices ») provenant de foies de donneurs. On espère que ces cellules ont un effet combiné systémique (dans tout le corps) et local dans le foie. Nous pensons qu'elles jouent un rôle immunomodulateur, c.-à-d. qu'elles aident à réguler la réponse inflammatoire exagérée qui est observée au cours de votre maladie, en particulier qu'elles aident à résoudre l'épisode aigu actuel de votre affection. Les cellules testées sont appelées « cellules progénitrices allogéniques (c.-à-d. provenant d'une autre personne) isolées à partir de foie humain adulte » ou HepaStem.
Les cellules sont des cellules souches isolées à partir de foies adultes et ne sont pas des cellules souches embryonnaires. Elles sont conservées selon une méthode spécifique et les autorités de santé ont approuvé leur utilisation chez des patients dans le cadre d'une étude clinique.

Douze (12) patients seront inclus dans cette étude en Europe, dont environ 6 en Belgique. Le médecin investigateur proposera à des patients qui, comme vous, présentent un diagnostic de cirrhose et une insuffisance hépatique aiguë sur maladie chronique du foie, de participer à cette étude.

L'objectif principal de cette étude est d'évaluer la sécurité et l'efficacité de deux (2) posologies d'HepaStem administrées 4 fois sur une période de 14 jours (1 administration tous les 3 jours).

Il s'agit d'une étude réalisée en ouvert pour évaluer 2 schémas posologiques, ce qui signifie que vous recevrez

- 4 administrations de la dose faible de cellules (250 millions de cellules pour chaque administration) sur une période de 14 jours (cela signifie l'administration d'1 seringue de 50 ml deux fois par semaine pendant 2 semaines), ou
- 4 administrations de la dose élevée de cellules (500 millions de cellules pour chaque administration) sur une période de 14 jours (cela signifie l'administration de 2 seringues de 50 ml deux fois par semaine pendant 2 semaines).

Dans les deux cas, vous et votre médecin investigateur saurez quelle posologie vous recevez.

Déroulement de l'étude

Après la phase de traitement de 2 semaines par les cellules et une période supplémentaire de 2 semaines de surveillance, vous resterez encore dans l'étude pendant 1 an (phase de suivi). Au cours du premier mois, vous ferez l'objet de 8 évaluations d'étude, c.-à-d. que vous subirez ces jours-là des procédures et des examens spécifiques à l'étude. Pendant la phase de suivi, vous ferez l'objet de 4 évaluations d'étude.

Les examens et procédures nécessaires liés à l'étude sont décrits ci-dessous. Certains de ces examens et procédures font partie des soins standards prodigués par votre hôpital, tandis que d'autres sont offerts dans le cadre de cette étude.

Votre participation à l'étude sera constituée de 3 phases :

1 Phase de sélection : pendant cette phase, votre médecin investigateur vérifiera si vous êtes éligible pour participer à cette étude, et établira un relevé de vos antécédents médicaux et des résultats de certains examens réalisés après avoir reçu votre accord pour participer à l'étude.

Tout au plus 25 ml supplémentaires de sang (environ 2 cuillères à soupe) seront prélevés. Des prises de sang sont déjà réalisées dans le cadre des soins liés à votre situation clinique actuelle ; aucune piqûre supplémentaire ne sera donc réalisée sur votre bras pour prélever ces tubes de sang.
Certaines analyses d'urine seront également réalisées.
Les échantillons de sang et d'urine permettront d'analyser des paramètres qui fourniront des informations sur l'état de vos organes (foie, reins, sang), sur votre statut virologique (notamment pour l'hépatite et l'infection à VIH) et sur votre immunité.
Si ce n'est pas déjà fait, on réalisera une échographie de votre foie et de votre cœur, une radiographie du thorax et un électrocardiogramme. Ces examens ne sont pas invasifs et ils sont indolores.
Si vous avez déjà subi une biopsie du foie pendant cette hospitalisation, les résultats de cet examen seront collectés.
Vous recevrez une « carte d'urgence » mentionnant toutes les informations sur l'étude. Veuillez porter cette carte sur vous en permanence. Si vous n'êtes pas hospitalisé(e), veuillez montrer cette carte à votre médecin et l'informer que vous participez à cette étude.

Après la phase de sélection, si vous répondez à tous les critères nécessaires pour entrer dans l'étude, vous serez en mesure de débuter la phase active de l'étude et vous recevrez le médicament HepaStem.

2 Phase active de l'étude : Pendant cette phase, vous recevrez 4 administrations d'HepaStem sur une période de 14 jours. En fonction du groupe auquel vous appartenez, vous recevrez 250 ou 500 millions de cellules par administration, ce qui équivaut à un volume de 50 ou 100 ml de liquide.

HepaStem sera administré par voie intraveineuse, ce qui signifie qu'une aiguille sera introduite dans une veine de petit ou grand calibre pour administrer le médicament. En vue de prévenir les réactions indésirables de votre corps au médicament, un médicament sera administré avant la perfusion d'HepaStem.

Pendant chaque administration et dans tous les cas, 2, 3 et 4 semaines après la 1ère administration, vous ferez l'objet d'examens cliniques et des échantillons de sang seront prélevés (environ 15 mL de sang soit 1 cuillère à soupe). Les échantillons de sang permettront d'obtenir des informations sur l'état de vos organes (foie, reins, sang).
Une échographie de votre cœur sera réalisée après la 1ère administration et une échographie de votre foie sera réalisée après avant chaque perfusion, puis 2 et 4 semaines après la 1ère administration.

3 Phase de suivi : pendant cette période, vous serez invité(e) à revenir à l'hôpital 2 mois, 3 mois, 6 mois et 12 mois après l'administration de la 1ère perfusion de cellules. Au cours de ces visites, vous ferez l'objet d'un examen clinique et un échantillon de sang sera prélevé (environ 15 mL de sang soit 1 cuillère à soupe). Les résultats de ces examens seront collectés.
Les échantillons de sang permettront d'analyser des paramètres qui fourniront des informations sur l'état de vos organes (foie, reins, sang).
Pendant cette période, si vous avez subi une transplantation du foie, un échantillon de votre foie explanté sera collecté si possible.

Après la phase de suivi, nous vous demanderons si vous acceptez d'être inscrit(e) dans notre registre dans le cadre d'un suivi régulier et complémentaire.

Risques et inconvénients

A : Effets secondaires du médicament de l'étude

- Tous les médicaments ont des effets secondaires connus ou imprévisibles. Même si l'étude précédente a révélé qu'HepaStem était bien toléré, il est toujours possible que vous présentiez les effets secondaires suivants : **à court terme :**
  - Thrombose
- Affection respiratoire Réaction d'hypersensibilité ou réaction à la perfusion : ceci se produit lorsque le système immunitaire du corps sur-réagit à quelque chose comme un médicament. La réaction d'hypersensibilité peut comprendre : irritation de la peau, rougeur, démangeaisons, gonflement, suintement, formation de croûtes, éruptions, toux ou essoufflement, enrouement de la voix, maux de tête, nez bouché ou qui coule, éternuements, yeux rouges, douleurs à l'estomac, nausée, vomissements, diarrhée, fatigue, mal de gorge, vertiges. Ces réactions peuvent être préjudiciables, inconfortables ou occasionnellement, fatales (en cas d'anaphylaxie). **à moyen ou long terme :**
  - distribution dans différents organes où les cellules peuvent favoriser le développement d'une tumeur, même si ces effets ont été rarement rapportés avec la thérapie cellulaire réaction

immunitaire car HepaStem est constitué de cellules provenant d'une autre personne, ce qui peut éventuellement induire une réaction de rejet des cellules.

D'autres risques et inconvénients inconnus à ce jour pourraient également apparaitre. Il est donc très important de signaler rapidement tout nouveau problème de santé au médecin investigateur, que vous pensiez ou non qu'il soit en rapport avec l'étude.

C : Risques associés aux procédures spécifiques à l'étude

Il existe également des risques/inconvénients associés aux examens spécifiques qui seront réalisés dans le cadre de cette étude :

- L'administration intraveineuse peut provoquer les effets suivants :
  - Douleur, comme pour toute injection.
  - Infection. Toute ouverture au niveau de la peau peut comporter un risque d'infection, même si l'insertion intraveineuse est une procédure aseptique.
  - Phlébite : inflammation d'une veine pouvant être causée par une infection.
  - Infiltration : une infiltration survient lorsqu'un liquide ou un médicament administré par voie intraveineuse pénètre accidentellement dans le tissu environnant plutôt que dans la veine.
  - Embolie : un caillot sanguin ou une autre particule solide, ou encore une bulle d'air, peut être introduit dans la circulation au cours de l'administration IV et bloquer finalement un vaisseau. Cependant, il est presque impossible d'injecter de l'air au cours d'une administration IV par voie périphérique. Le risque est plus élevé en cas d'administration IV par voie centrale.
- La **prise de sang** nécessaire aux analyses peut (rarement) causer une douleur, un saignement, une contusion (bleu) ou une infection autour du site d'injection. Chez certains patients, des étourdissements, ou même un évanouissement, peuvent survenir pendant la procédure.

L'équipe qui réalisera la prise de sang fera usage des meilleurs soins médicaux pour empêcher ou réduire au minimum ces inconforts.

Notification de nouvelles informations

Il est possible que, pendant le déroulement de l'étude clinique, de nouvelles informations importantes sur HepaStem, le médicament étudié, deviennent disponibles. Vous serez informé(e) au moment opportun de tout élément nouveau susceptible de modifier votre décision de poursuivre votre participation à cette étude.

Si, au vu de ces nouvelles informations, vous décidez d'interrompre votre participation à l'étude, votre médecin investigateur veillera à ce que vous continuiez à recevoir le meilleur traitement possible.

Contraception, grossesse et allaitement

Participant de sexe féminin : Étant donné que les effets d'HepaStem sur un enfant à naître ou un nourrisson ne sont pas parfaitement connus, vous ne serez pas autorisée à participer à cette étude clinique si vous êtes enceinte, si vous souhaitez tomber enceinte ou si vous allaitez.

Si vous choisissez de participer à cette étude, vous devrez utiliser l'une des méthodes contraceptives autorisées (de manière à ne pas tomber enceinte). Votre médecin discutera avec vous des différentes options adéquates.

Bénéfices

Si vous acceptez de participer à cette étude, HepaStem pourra ou non s'avérer bénéfique pour le traitement de la maladie dont vous êtes atteint(e) ou soulager vos symptômes, en particulier pour aider à résoudre la décompensation aiguë actuelle de la fonction de votre foie.

Les informations obtenues grâce à cette étude peuvent contribuer à une meilleure connaissance de l'utilisation de ce médicament ou au développement d'un nouveau médicament pour le traitement de l'insuffisance hépatique aiguë sur maladie chronique du foie chez de futurs patients.

Traitement alternatif :

À ce jour, aucun traitement de votre affection approuvé par les autorités n'est disponible en Europe.

Le seul traitement actuellement disponible est la greffe de foie. Cependant, ce traitement est loin d'être idéal vu le manque d'organes pour la transplantation.

Retrait de l'étude

Votre participation est volontaire et vous avez le droit de vous retirer de l'étude à tout moment et sans devoir vous justifier. Néanmoins, il peut être utile pour le médecin investigateur et pour le promoteur de l'étude de savoir si vous vous retirez de l'étude car les contraintes liées au traitement sont trop importantes (par exemple, trop d'effets secondaires désagréables).

Il est aussi possible que le médecin investigateur vous retire de l'étude car vous êtes enceinte, car il/elle pense que c'est mieux pour votre santé ou car il/elle constate que vous ne respectez pas les consignes données aux participants.

Enfin, il est également possible que les autorités compétentes nationales ou internationales, le comité d'éthique qui a initialement approuvé l'étude ou le promoteur, décident d'interrompre l'étude.

Si vous retirez votre consentement à participer à l'étude, afin de garantir la validité de la recherche, les données encodées jusqu'au moment du retrait, seront conservées. Aucune nouvelle donnée ne sera envoyée au promoteur.

Si vous retirez votre consentement à participer à l'étude, vous pouvez contacter l'investigateur et demander que vos échantillons qui n'ont pas encore été utilisés, soient détruits. Les résultats obtenus de vos échantillons avant le retrait de votre consentement, restent la propriété du promoteur de l'étude.

Traitement après l'arrêt de l'étude

Dans toutes ces situations de retrait de l'étude mais également lorsque la période de participation prévue est arrivée à son terme, votre médecin investigateur évaluera votre état de santé et vous prescrira le meilleur traitement disponible.

Échantillons de matériel biologique collectés au cours de l'étude

Le promoteur de l'étude s'engage à ce que les échantillons (p. ex. sang, urine, tissu hépatique) ne soient utilisés que dans le cadre de l'étude.

- La procédure de codage des échantillons est la même que celle utilisée pour vos données médicales. Les échantillons envoyés au promoteur ne comprendront donc que votre code ID de l'étude.

- Le gestionnaire de ces échantillons (laboratoire d'hématologie des Cliniques St Luc, Bruxelles et Translational Research Center, KU Leuven, Louvain) s'engagent à les utiliser dans le contexte de la recherche clinique et de les détruire à la fin de la période de stockage prévue.

- L'échantillon de matière biologique prélevé est considéré comme un « don » et vous devez savoir que, en principe, vous ne recevrez aucun avantage financier (royalties) associé au développement d'un nouveau traitement dérivé de l'utilisation de votre don de matière biologique, qui pourrait présenter une valeur commerciale.

Le surplus de vos échantillons sera détruit dès que les analyses décrites dans ce document auront été réalisées (au plus tard, un an après la fin de l'étude).

Confidentialité et protection des données

Votre participation à l'étude signifie que vous consentez à la collecte de données à votre sujet par l'investigateur et à l'utilisation de ces données par le promoteur à des fins de recherches et en lien avec des publications scientifiques et médicales.
Vous avez le droit de demander à l'investigateur quelles données sont collectées à votre sujet et quelle est leur utilisation dans le cadre de l'étude. Ces données concernent votre état clinique actuel, mais également une partie de vos antécédents, les résultats des examens réalisés dans le contexte de votre prise en charge selon les standards actuels et évidemment, les résultats des examens requis par le protocole. Vous avez le droit de consulter ces données et de les corriger si elles sont incorrectes. Ces droits sont garantis par la loi du 8 décembre 1992 relative à la protection de la vie privée à l'égard du traitement de données à caractère personnel et par la loi du 22 août 2002 relative aux droits du patient.
L'investigateur a un devoir de confidentialité vis-à-vis des données collectées.
Cela signifie qu'il/elle s'engage, non seulement à ne jamais révéler votre nom dans le contexte d'une publication ou d'une conférence, mais également qu'il/elle codera (votre identité sera remplacée par un code ID dans l'étude) vos données avant de les envoyer au gestionnaire de la banque des données collectées (Clinical Department, Promethera Biosciences).
L'investigateur et son équipe seront donc les seules personnes capables d'établir un lien entre les données transmises au cours de l'étude et votre dossier médical. Pour la présente étude, la loi exige que ce lien avec votre dossier soit conservé pendant minimum 30 ans et maximum 50 ans, loi belge du 19 décembre 2008 relative à l'obtention de matériel biologique humain et les arrêtés royaux qui s'y rapportent.
Les données personnelles transmises ne contiendront aucune combinaison d'éléments qui pourraient permettre de vous identifier.
Pour le gestionnaire des données de l'étude désigné par le promoteur, les données transmises ne permettront pas de vous identifier. Ce dernier est responsable de la collecte des données recueillies par tous les investigateurs participant à l'étude, leur traitement et leur protection selon les exigences de la loi belge relative à la protection de la vie privée.
Afin de vérifier la qualité de l'étude, il est possible que vos dossiers médicaux soient examinés par des personnes soumises au secret professionnel et désignées par le comité d'éthique, le promoteur de l'étude ou un audit indépendant. Dans tous les cas, l'examen de votre dossier médical sera sous l'entière responsabilité de l'investigateur et sous la supervision de l'un de ses collaborateurs, qu'il/elle aura désigné.
Les données de l'étude (codées) pourront être envoyées à des autorités de règlementation belges ou autres, aux comités d'éthique pertinents, à d'autres médecins et/ou à des organisations travaillant en collaboration avec le promoteur.
Elles pourront également être envoyées vers d'autres sites du promoteur en Belgique et dans d'autres pays où les standards en termes de protection des données personnelles peuvent être différents ou moins rigoureux. Comme indiqué ci-dessus, les données transmises sont codées. Le promoteur s'engage donc au respect des contraintes de la directive européenne et de la législation belge sur la protection de la vie privée.
Votre consentement à participer à la présente étude implique par conséquent également, votre consentement à l'utilisation de vos données médicales codées pour les objets décrits dans le présent feuillet d'information et à leur transmission aux personnes et autorités mentionnées ci-dessus.
Le promoteur s'engage à n'utiliser les données collectées que dans le contexte de l'étude à laquelle vous participez.

Assurance

Toute participation à une étude clinique comprend des risques, aussi faibles soient-ils. Même si aucune erreur n'a été commise, le promoteur assume la responsabilité de tout dommage causé au

participant (ou dans le cas d'un décès, ses ayants-droits) et lié directement ou indirectement à sa participation à l'étude. Le promoteur a souscrit une assurance couvrant cette responsabilité.
Il vous est donc demandé d'informer l'investigateur de tout nouveau problème de santé avant de consulter un autre médecin, de prendre un autre médicament ou de recevoir tout autre traitement médical. Si pour une raison quelconque, vous consultez un autre médecin pendant la présente étude clinique, vous devez l'informer que vous participez à une étude clinique/lui présenter votre carte de participant à une étude clinique. Ceci peut être important pour établir un diagnostic et traiter vos plaintes.
Si l'investigateur pense d'un lien avec l'étude est possible (l'assurance ne couvre pas la progression naturelle de votre maladie ou les effets secondaires connus de votre traitement normal), il/elle en informera le promoteur de l'étude, qui initiera la procédure de déclaration à la compagnie d'assurance. Cette dernière nommera un expert – si elle le considère nécessaire – pour établir s'il existe un lien entre votre nouveau problème de santé et l'étude.
Dans le cas d'un désaccord avec l'investigateur ou l'expert désigné par la compagnie d'assurance, et également si vous en ressentez la nécessité, vous ou – en cas de décès - vos ayants-droits pouvez entamer une procédure à l'égard de l'assureur, directement en Belgique (nom de la compagnie d'assurance, numéro de la police, contact).
La loi assure que l'assureur peut être convoqué par le juge de l'endroit où l'événement donnant lieu au dommage s'est déroulé, ou devant le juge de votre domicile, ou devant le juge du siège social de l'assureur.

Si vous participez à cette étude clinique, nous vous demandons :

- De ne pas boire d'alcool pendant au moins les 5 semaines suivant le début de votre participation.
- De collaborer pleinement au bon déroulement de cette étude.
- De ne dissimuler aucune information relative à votre état de santé, aux médicaments que vous prenez ou aux symptômes que vous présentez.
- De ne participer à aucune autre étude clinique impliquant un traitement expérimental (médicament, dispositif médical ou procédure) pendant votre participation à cette étude.
- De porter en permanence sur vous la « carte d'urgence ». Cette précaution est indispensable pour garantir votre sécurité si vous deviez recevoir des soins d'urgence dans une institution qui ne vous connaît pas.

Contact

Si vous avez besoin d'informations supplémentaires, mais aussi en cas de problème ou d'inquiétude, vous pouvez contacter le médecin investigateur …… ou un membre de son équipe de recherche …… au numéro de téléphone suivant (xx / xxx-xx-yy) ……..

En cas d'urgence ou en-dehors des heures de consultation, vous pouvez contacter le service des urgences …… au numéro de téléphone suivant ……..., en mentionnant que vous participer à une étude clinique. Votre dossier contiendra les informations sur cette étude clinique, qui seront utiles pour le médecin de garde.

ÉTUDE HEP101

Étude Clinique multicentrique de phase II visant à évaluer la sécurité et l'efficacité préliminaire de 2 doses d'HepaStem chez des patients souffrant d'insuffisance hépatique aiguë sur une maladie chronique du foie.

Consentement éclairé du patient

ID du patient de l'étude : ……..

Participant

Je déclare que j'ai été informé(e) sur la nature de l'étude, son but, sa durée, les éventuels bénéfices et risques et ce que l'on attend de moi. J'ai pris connaissance du document d'information. J'ai eu suffisamment de temps pour y réfléchir et en parler avec une personne de mon choix, p. ex. mon médecin généraliste (MG) ou un membre de ma famille.

J'ai eu l'occasion de poser au médecin investigateur toutes les questions qui me sont venues à l'esprit et j'ai obtenu une réponse satisfaisante à mes questions.

Je comprends que ma participation à cette étude est volontaire et que je suis libre de mettre fin à ma participation à cette étude sans que cela ne modifie mes relations avec l'équipe thérapeutique en charge de ma santé.

Je comprends qu'au cours de cette étude clinique, mes données personnelles (en particulier mon dossier médical) seront récoltées, conservées et analysées. L'utilisation des informations relatives à ma santé est conforme aux dispositions légales et nécessite un consentement éclairé et volontaire préalable à ma participation à cette étude. Sans avoir signé le formulaire de consentement, je ne peux pas participer à l'étude.
Les données personnelles sont codées. J'ai le droit de consulter/corriger ces données.

Le consentement à la collecte et au traitement de mes données personnelles, en particulier des informations relatives à ma santé, est irrévocable. J'ai déjà été informé(e) que je peux mettre un terme à ma participation à cette étude à tout moment. Dans le cas où je retire mon consentement, je consens à ce que les données collectées jusqu'à ce moment-là puissent être analysées de manière pseudonyme (codées)

J'accepte que mes données soient conservées après la fin de l'étude ou mon interruption de l'étude, conformément aux exigences nationales requises.

J'accepte que les données d'étude collectées en vue d'atteindre les objectifs de cette étude puissent être traitées ultérieurement, pour autant que ce traitement soit limité au cadre de la présente étude pour une meilleure connaissance de la maladie et de son traitement.

J'accepte que le promoteur conserve des échantillons de matériel biologique récoltés en cours de l'étude après la fin de l'étude clinique, à des fins de recherches ultérieures mais limitées au cadre de la présente étude.

J'accepte que mon MG ou d'autres spécialistes en charge de ma santé soient informés de ma participation à cette étude clinique.

J'ai reçu une copie du document d'information du participant et du formulaire de consentement éclairé.

Prénom du patient : ……Nom du patient : ……

Signature : …… Date : ……

Représentant légal

Je déclare que j'ai été invité(e) à confirmer la décision sur la participation éventuelle à l'étude clinique de la personne que je représente, en tenant compte de ses intérêts et de sa probable volonté. Mon consentement s'applique à tous les points repris dans le formulaire de consentement du participant.

J'ai reçu une copie du document d'information du participant et du formulaire de consentement éclairé.

Prénom du représentant légal : ……Nom du représentant légal :……

Signature : …… Date : …….. ;……

Témoin/interprète

J'ai été présent pendant l'entièreté du processus d'information du patient et je confirme que les informations relatives aux objectifs et procédures de l'étude ont été fournies de manière adéquate, que le participant (ou son représentant légal) a apparemment compris l'étude et que le consentement à participer à l'étude a été donné librement.

Prénom et nom du témoin/de l'interprète : ……

Signature : …… Date : ……

Médecin investigateur

Je soussigné(e),....., médecin investigateur, confirme avoir fourni verbalement les informations nécessaires sur l'étude et avoir fourni une copie du document d'information au participant.

Je confirme qu'aucune pression n'a été exercée pour persuader le patient d'accepter de participer à l'étude et que je suis prêt(e) à répondre à toutes les questions supplémentaires si cela s'avère nécessaire.

Je confirme travailler en accord avec les principes éthiques énoncés dans la dernière version de la « Déclaration d'Helsinki », des « Bonnes pratiques Cliniques » et de la loi nationale en vigueur relative aux expérimentations sur la personne humaine.

Prénom du médecin investigateur : ……Nom du médecin investigateur : ……

Signature : …… Date : ……

ÉTUDE HEP101

ETUDE CLINIQUE MULTICENTRIQUE DE PHASE II VISANT À ÉVALUER LA SÉCURITÉ ET L'EFFICACITÉ PRÉLIMINAIRE DE 2 DOSES D'HEPASTEM CHEZ DES PATIENTS SOUFFRANT D'INSUFFISANCE HÉPATIQUE AIGUË SUR UNE MALADIE CHRONIQUE DU FOIE.

Information rétrospective

En raison de votre situation clinique au moment de votre inclusion dans l'étude mentionnée ci-dessus, vous n'étiez pas pleinement capable de décider vous-même si vous souhaitiez participer on non à cette étude.

Il est alors habituel d'utiliser un représentant légal (généralement un proche), à qui l'on demande de confirmer la participation éventuelle d'une personne à l'étude en tenant compte de ses intérêts et de sa probable volonté.

Vous devez savoir que le comité d'éthique (de Louvain) a accepté l'application de cette procédure après consultation des comités d'éthique d'autres centres participants le …….

Vous avez donc été inclus(e) dans cette étude, vous étiez d'accord pour participer, mais, n'étant pas complétement capable de décider vous-même, le médecin investigateur a demandé à votre représentant légal de confirmer votre participation en signant également le consentement.

Désormais, l'amélioration de votre situation clinique vous permet d'être correctement informé(e) sur les objectifs et procédures de l'étude et de donner votre consentement à ce sujet et vous permet de décider de poursuivre votre participation dans l'étude ou de l'interrompre.

Nous vous demandons à présent de confirmer votre souhait de poursuivre cette participation ou de l'interrompre, et nous vous invitons à lire le document vous fournissant des informations complètes sur les objectifs et procédures de l'étude mais aussi sur les risques et bénéfices possibles liés au médicament expérimental et sur vos droits en tant que participant à une étude clinique.

Veuillez lire attentivement ces quelques pages d'information et poser toutes les questions que vous souhaitez au médecin investigateur ou à son représentant. Ce document comprend 2 parties : l'information essentielle à votre prise de décision et votre formulaire de consentement écrit.

Dans ce cas de figure, un nouveau consentement éclairé sera re-signé par vous ainsi que par le médecin investigateur de l'étude.

HEP101 STUDIE

MULTICENTRISCHE KLINISCHE FASE-2-STUDIE NAAR DE VEILIGHEID EN VOORSTUDIE NAAR DE WERKZAAMHEID VAN TWEE DOSERINGSREGIMES VAN HEPASTEM BIJ PATIËNTEN MET ACUUT LEVERFALEN BIJ EEN CHRONISCHE LEVERZIEKTE

Patiënteninformatie

Deze patiënteninformatie en dit formulier voor geïnformeerde toestemming zijn bedoeld voor patiënten die lijden aan acuut-op-chronisch leverfalen en de wettelijke vertegenwoordigers van deze patiënten die worden uitgenodigd om aan een wetenschappelijk onderzoek met HepaStem deel te nemen

Studiesponsor: **Promethera Biosciences**
Adres: **Rue Granbonpré 11**
1435 Mont St Guibert
België

Voornaamste onderzoeker: _____
Adres _____
Telefoonnummer: _____

Introductie

U wordt uitgenodigd om deel te nemen aan een klinische studie met het oog op de evaluatie van een medisch onderzoeksproduct voor de behandeling van uw ziekte. Een medisch onderzoeksproduct is een medisch product dat nog steeds wordt bestudeerd om de effectiviteit, veiligheid en werking ervan te evalueren.

Aan de patiënt: het is mogelijk dat u op het moment dat u in de studie wordt opgenomen, niet helemaal in staat bent om zelf te beslissen of u al dan niet aan deze studie wenst deel te nemen. In dat geval is het gebruikelijk om een wettelijke vertegenwoordiger in te schakelen, aan wie wij vragen om uw beslissing over uw deelname te bevestigen, rekening houdend met uw belangen en uw waarschijnlijke wens.

Aan de wettelijke vertegenwoordiger: we denken dat de persoon die u vertegenwoordigt, in staat is te beslissen om al dan niet aan deze studie deel te nemen. Het is echter mogelijk dat hij/zij zich wegens zijn/haar ziekte niet helemaal bewust is van de gevolgen in verband met de studie. Daarom vragen wij u om te bevestigen of de persoon die u vertegenwoordigt al dan niet aan deze klinische studie wenst deel te nemen, rekening houdend met zijn/haar belangen en zijn/haar waarschijnlijke wens.

Het vervolg van dit document is opgesteld alsof het rechtstreeks gericht is aan de persoon die u vertegenwoordigt.

De Onderzoeker (of de studiearts is de arts die verantwoordelijk is voor deze wetenschappelijke studie in het ziekenhuis) en de Sponsor (bedrijf dat het wetenschappelijk onderzoek start en financiert) hopen dat dit medisch product helpt bij de behandeling van patiënten met dezelfde ziekte als de uwe. Er is echter geen waarborg dat uw deelname aan deze studie uw gezondheid ten goede komt.

Vooraleer u instemt om aan deze studie deel te nemen, vragen we u om rekening te houden met de implicaties op het vlak van organisatie, mogelijke risico's en voordelen, wat u in staat moet stellen om, volledig bewust van de implicaties, een beslissing te nemen. Dit is gekend als het geven van een "geïnformeerde toestemming".

Gelieve deze informatie nauwkeurig te lezen en leg alle vragen waarmee u zit voor aan de onderzoeker of zijn/haar vertegenwoordiger. Dit document bestaat uit 2 delen: de informatie die essentieel is voor uw beslissing en, het ingevulde en ondertekende toestemmingsformulier.

Vooraleer u beslist, moet u bewust zijn van het volgende:

- Deze klinische studie wordt uitgevoerd nadat ze door een ethisch comité (in Leuven) werd beoordeeld na overleg met de ethische comités van andere deelnemende centra.
- Uw deelname is vrijwillig en moet verder zonder enige dwang verlopen. Een ondertekening van een document waarin u uw toestemming te kennen geeft, is vereist. Zelfs nadat u dit document hebt ondertekend, kunt u uw deelname stopzetten door de onderzoeker hierover te informeren. Uw beslissing om niet deel te nemen zal geen impact hebben op de kwaliteit van de zorg of op uw relatie met de onderzoeker.
- De gegevens die in deze studie worden verzameld zijn vertrouwelijk en uw anonimiteit is altijd gewaarborgd, ook tijdens de publicatie van de resultaten.
- De sponsor heeft een polis getekend bij een verzekeringsmaatschappij, ingeval u enige schade zou oplopen in verband met uw deelname aan deze studie.

  Verzekeringsmaatschappij: QBE Syndicate

  Polisnummer: 16ME305419EA
- Uw deelname aan de studie is volledig gratis. U zult geen kosten aangerekend krijgen voor de onderzoeken die specifiek zijn voor deze studie. U zult echter ook niet betaald worden voor uw deelname aan deze studie.
- Uw onkosten voor uw vervoer naar het ziekenhuis (bv. de kosten voor uw auto, taxi, treinticket, enz.) zullen u vergoed worden met waardebonnen (1 waardebon/uitgevoerd onderzoeksbezoek na uw ontslag uit het ziekenhuis). Neem contact op met het studieteam voor praktische informatie.
- De sponsor zal het ziekenhuis/de onderzoeker vergoeden voor alle kosten voor bezoeken/consultaties, onderzoeken of behandelingen die specifiek zijn voor deze studie.
- U mag de onderzoeker contacteren of een lid van zijn/haar team, telkens wanneer u bijkomende informatie wenst.

Doelstellingen en beschrijving van het studieprotocol

Wij nodigen u uit om deel te nemen aan een klinische studie waarbij gebruik wordt gemaakt van menselijke levercellen (progenitorcellen genoemd) afkomstig van gedoneerde levers. Van deze cellen verwacht men dat ze een gecombineerd systemisch (in heel uw lichaam) en lokaal effect op de lever hebben. Men verwacht dat ze een immunomodulerende functie hebben, wat betekent dat ze de overdreven, ontstekingsreactie die bij uw ziekte werd vastgesteld helpen regelen en vooral een oplossing bieden voor het huidige acute event. De cellen die worden getest worden heterologe (van een andere persoon) progenitorcellen genoemd, afkomstig van de lever van een volwassen persoon (HHALPC) of HepaStem.

De cellen zijn stamcellen, die uit volwassen levers zijn geïsoleerd en ze zijn geen embryonale stamcellen. Ze worden op een specifieke wijze bewaard en zijn goedgekeurd door de gezondheidsinstanties om te worden gebruikt bij patiënten in het kader van een klinisch onderzoek.

Twaalf (12) patiënten zullen in Europa in deze studie worden opgenomen, waaronder ongeveer 6 in België. De onderzoeker zal aan patiënten voorstellen om deel te nemen, waarbij zoals bij u, een diagnose van levercirrose en acuut op chronisch leverfalen werd vastgesteld.

Het voornaamste doel van deze studie bestaat erin om de veiligheid en effectiviteit te beoordelen van twee (2) doseringen van HepaStem 4 maal toegediend in een periode van 14 dagen (om de 3 dagen 1 keer toegediend).

Deze studie is een open studie van 2 doseringsregimes. Dit betekent dat u ofwel

- 4 x een lage dosis cellen (250 miljoen cellen elk) in een periode van 14 dagen krijgt toegediend (dit betekent de toediening van 1 spuit van 50 ml tweemaal per week gedurende 2 weken) of
- 4 x een hoge dosis cellen (500 miljoen cellen in een periode van 14 dagen) (dit betekent de toediening van 2 spuiten van 50 ml tweemaal per week gedurende 2 weken)

In beide gevallen zullen u en uw onderzoeker op de hoogte zijn van de dosis die u zal worden toegediend.

Verloop van de studie

Na een behandeling van 2 weken met cellen en nog eens 2 weken tijdens de observatieperiode, zal u in de studie blijven tot 1 jaar voorbij is (follow-up periode). Binnen de eerste maand zal u 8 studiebeoordelingen krijgen, d.w.z. dagen met procedures en onderzoeken die specifiek aan de studie gekoppeld zijn. Tijdens de follow-up periode zal u 4 studiebeoordelingen krijgen.

De onderzoeken en procedures die in verband met de studie nodig zijn, worden hieronder beschreven. Enkele onderzoeken en procedures maken deel uit van de standaardzorg die in het ziekenhuis wordt verleend, terwijl anderen in het kader van de studie worden aangeboden.

Uw deelname aan de studie zal over 3 periodes verlopen:

1 Screeningsperiode: in die periode zal uw onderzoeker verifiëren of u in aanmerking komt om aan de studie deel te nemen en hij zal uw medische geschiedenis en de gegevens vergaren van enkele onderzoeken die werden gedaan, nadat u had toegestemd om aan de studie deel te nemen.

Er zullen ten hoogste 25 mL bloed (ongeveer 2 eetlepels bloed) worden afgenomen. Bloedstalen zijn reeds genomen in het kader van de zorgverlening in uw huidige klinische situatie; daarom zal geen bijkomende prik in uw arm worden gegeven om deze buisjes bloed te verzamelen.

Er zal ook een analyse van uw urine plaatsvinden.
De bloed- en urinestalen zullen ons toelaten om de parameters te analyseren die ons meer zullen zeggen over de staat van uw organen (lever, nieren, bloed), over uw virologische status (waaronder hepatitis en HIV) alsook uw immuniteit.
Indien het nog niet is gebeurd, zal een echografie worden genomen van uw lever en hart, X-stralen van uw borstkas en een electrocardiogram. Deze onderzoeken zijn niet-invasieve behandelingen en doen geen pijn.
Indien u tijdens uw opname in het ziekenhuis een leverbiopsie hebt gehad, zullen de resultaten van dit onderzoek worden verzameld.
U zal een “noodkaart” met informatie over deze studie ontvangen. Houd deze kaart steeds bij u. Indien u in het ziekenhuis wordt opgenomen, toon dan die kaart aan uw arts en informeer hem/haar dat u aan deze studie deelneemt.

Na de screeningsperiode, zal u, nadat aan alle criteria is voldaan om aan deze studie deel te nemen, in staat zijn om de actieve periode in de studie aan te vangen en HepaStem te krijgen.

2_ Actieve periode: Tijdens deze periode zal u in de loop van 14 dagen 4 x HepaStem toegediend krijgen. Afhankelijk van de groep waartoe u behoort, zal u 250 of 500 miljoen cellen worden toegediend. Dit beantwoordt aan een volume van 50 of 100 mL vloeistof.

HepaStem zal intraveneus worden toegediend, wat betekent dat een naald in een kleine of grote ader zal worden geplaatst om het product toe te dienen. Voorafgaand aan de HepaStem infusie zal medicatie worden gegeven om slechte reacties van het lichaam op het product te voorkomen.

Tijdens het toedienen en in elk geval 2, 3 en 4 weken na de eerste toediening, zal u klinische onderzoeken ondergaan en zullen er bloedstalen worden genomen (max 15mL bloed, ongeveer 1 eetlepels bloed).
De bloedstalen zullen toelaten om informatie in te winnen over de status van uw organen (lever, nieren, bloed).
Er zal een echografie van uw hart worden gemaakt na de eerste toediening en een echografie van uw lever voor elke infusie, 2 en 4 weken na de eerste toediening.

3_ Follow-up periode: tijdens deze periode, zullen we u vragen om 2 maanden, 3 maanden, 6 maanden en 12 maanden na de eerste celinfusie naar het ziekenhuis terug te komen. Bij deze bezoeken zal u een klinisch onderzoek ondergaan en een bloedstaal (max 15mL bloed, ongeveer 1 eetlepels bloed). De resultaten van deze onderzoeken zullen worden verzameld.
De bloedstalen zullen ons toelaten om de parameters te analyseren die ons informatie zullen schenken over de status van uw organen (lever, nieren, bloed).
Indien u tijdens die periode een levertransplantatie hebt, zal indien mogelijk, een staal van uw verwijderde lever worden verzameld.

Na de follow-up periode, zullen we u vragen of u wilt instemmen om deel uit te maken van ons registratiesysteem voor bijkomende en regelmatige follow-up.

Risico's en ongemakken

A: Bijwerkingen van het medicijn van deze studie

- Alle medicijnen hebben gekende of onvoorspelbare bijwerkingen. Ook wanneer een voorafgaande studie heeft aangetoond dat HepaStem goed werd verdragen, kunt u toch de volgende bijwerkingen ervaren: **op korte termijn:**
  - trombose
- Ademhalingsmoeilijkheden overgevoeligheidsreactie of een reactie op de infusie: dit treedt op wanneer het immuunsysteem van het lichaam overmatig reageert op iets als medicatie. Overgevoeligheidsreacties kunnen omvatten: huidirritatie, roodheid, jeuk, zwelling, vochtafscheiding, korstvorming, huiduitslag, erupties, hoesten of kortademigheid, schorre stem, hoofdpijn, verstopte of lopende neus, niezen, rode (bloeddoorlopen) ogen, maagpijn, misselijkheid, braken, diarree, vermoeidheid, keelpijn, duizeligheid. Deze reacties kunnen schadelijk, ongemakkelijk of in sommige gevallen dodelijk zijn (in geval van anafylaxie): **op middellange of lange termijn:**
  - verspreiding in verschillende organen waar cellen de ontwikkeling van tumoren in de hand kunnen werken, hoewel dergelijke events zelden i.v.m. celtherapie werden gerapporteerd, een immuniteitsreactie aangezien HepaStem bestaat uit cellen van een andere persoon, wat eventueel tot afstoting van cellen kan leiden.

Andere risico's en ongemakken die voor het ogenblik niet bekend zijn, zouden zich kunnen voordoen. Het is daarom heel belangrijk dat elk nieuw gezondheidsprobleem vlug aan de onderzoeker wordt gerapporteerd, ongeacht of u al dan niet denkt dat het met de studie te maken heeft.

C: Risico's in verband met procedures die specifiek zijn voor deze studie

Er zijn ook risico's/ ongemakken, die verband houden met specifieke onderzoeken die in verband met deze studie zullen worden uitgevoerd:

- De intraveneuze toediening kan het volgende veroorzaken:
  - Pijn, zoals voor elke injectie.
  - Infectie. Elke opening in de huid houdt een risico van infectie in, hoewel intraveneuze injectie een aseptische procedure is.
  - Flebitis: ontsteking van een ader, die door infectie kan worden veroorzaakt.
  - Infiltratie: infiltratie doet zich voor wanneer een intraveneuze vloeistof of medicatie per toeval in het omgevende weefsel eerder dan in de ader terechtkomt.
  - Embolie: een bloedklonter of vaste massa, zoals ook een luchtbel, kan door een IV in de bloedsomloop terechtkomen en een bloedvat blokkeren. Evenwel, het is bijna onmogelijk om op een gevaarlijke manier lucht door een perifere IV te injecteren. Het risico is groter met een centrale IV.
- Het **afnemen van bloed,** noodzakelijk, voor een analyse kan (zelden) pijn veroorzaken, bloeden, blauwe plekken of infectie bij de plaats van de injectie tot gevolg hebben. Ook kunnen patiënten zich tijdens de procedure duizelig voelen of ze kunnen flauw vallen.

Het personeel dat bloed afneemt, zal de beste medische zorgen toedienen om ongemakken te voorkomen of ze tot een minimum te herleiden.

Bekendmaking van nieuwe informatie

Het kan gebeuren dat belangrijke nieuwe informatie bij het onderzoek m.b.t. HepaStem ter beschikking komt te staan. U zal op tijd worden geïnformeerd over elk nieuw element dat uw beslissing kan beïnvloeden om verder aan deze studie deel te nemen.

Indien u, in het licht van de nieuwe informatie, beslist om verder aan de studie deel te nemen, zal uw onderzoeker ervoor zorgen dat u verder de beste behandeling krijgt.

Contraceptie, zwangerschap en borstvoeding

Vrouwelijke deelnemer**:** omdat de gevolgen van HepaStem op een ongeboren kind of zuigeling niet echt gekend zijn, zal u niet worden toegelaten om aan deze klinische studie deel te nemen, wanneer u zwanger bent, wenst zwanger te worden of indien u borstvoeding geeft.

Indien u aan deze studie wenst deel te nemen, moet u één van de goedgekeurde contraceptiemethodes gebruiken (zodat u niet zwanger wordt). Uw arts zal de verschillende geschikte opties met u bespreken.

Baten

Indien u akkoord gaat om aan deze studie deel te nemen, kan HepaStem al dan niet blijken een gunstige werking te hebben bij de behandeling van uw ziekte of bij het verlichten van de symptomen, vooral bij het helpen oplossen van de huidige acute decompensatie van de leverfunctie.

De informatie die men dankzij deze studie krijgt, kan ertoe bijdragen om een betere kennis te krijgen van het gebruik van dit medisch product of van de ontwikkeling van een nieuw medisch product voor de behandeling van acuut-op chronisch leverfalen bij toekomstige patiënten.

Alternatieve behandeling:

Geen enkele door de overheden goedgekeurde behandeling van uw toestand, is voor het ogenblik in Europa beschikbaar.

De enige behandeling die nu beschikbaar is, is levertransplantatie. Evenwel, deze behandeling is ver van ideaal, wegens gebrek aan transplantatieorganen.

Terugtrekking uit de studie

Uw deelname is vrijwillig en u hebt het recht om u te allen tijde uit de studie terug te trekken, zonder dat u een reden opgeeft. Evenwel, het kan voor de onderzoeker en de sponsor van de studie nuttig zijn om te weten of u zich terugtrekt wegens de te grote last veroorzaakt door de behandeling (te veel onaangename bijwerkingen, bijvoorbeeld).

Het is ook mogelijk dat de onderzoeker u uit de studie haalt, omdat u zwanger bent, omdat hij/zij denkt dat het beter is voor uw gezondheid of omdat hij/zij er achter is gekomen dat u de instructies die aan de deelnemers werden gegeven niet opvolgt.

Tenslotte kunnen de bevoegde nationale of internationale overheden, het ethisch comité dat de studie eerst goedkeurde of de sponsor beslissen om de studie stop te zetten.

Als u uw toestemming om deel te nemen aan de studie intrekt, dan zullen, om de geldigheid van het onderzoek te garanderen, de gecodeerde gegevens tot het punt waarop u zich terugtrok, bewaard blijven. Er mogen geen nieuwe gegevens naar de sponsor verzonden worden.

Als u uw goedkeuring om deel te nemen aan de studie intrekt, kunt u contact opnemen met de onderzoeker en vragen om de stalen die nog niet gebruikt zijn, te laten vernietigen. De resultaten verkregen uit uw stalen voordat u uw goedkeuring introk, blijven eigendom van de studiesponsor.

Behandeling nadat de studie werd stopgezet

In al deze situaties van terugtrekking uit de studie, maar ook wanneer de geplande periode van uw deelname is beëindigd, zal uw onderzoeker de staat van uw gezondheid onderzoeken en de best beschikbare behandeling voorschrijven.

Stalen van biologisch materiaal dat gedurende de studie werd verzameld

De sponsor van deze studie verzekert dat de stalen (zoals bloed, urine, weefsel van de lever) alleen in het kader van de studie zullen worden gebruikt.

- De procedure voor het coderen van stalen is dezelfde als deze die wordt gebruikt voor uw medische gegevens. Stalen die naar de sponsor worden verstuurd, zullen daarom alleen uw studie-ID-code bevatten.
- De beheerder van deze stalen (het hematologielaboratorium van de Cliniques St. Luc in Brussel en het Translational Research Center, KU Leuven, Leuven) zal ze gebruiken binnen de context van klinisch onderzoek en ze vernietigen aan het eind van de geplande bewaarperiode.
- Het staal van afgenomen biologisch materiaal wordt beschouwd als een ‘donatie’ en u moet er zich van bewust zijn dat u, in principe, geen enkel financieel voordeel (royalty’s) zult ontvangen geassocieerd met de ontwikkeling van nieuwe therapieën die zijn afgeleid van het gebruik van uw donatie van biologisch materiaal en die mogelijk commerciële waarde kunnen hebben.

Het overschot van stalen zal worden vernietigd zodra de analyses die in dit document zijn beschreven, werden uitgevoerd (ten laatste, één jaar na beëindiging van de studie)

Vertrouwelijkheid en gegevensbescherming

Uw deelname aan de studie betekent dat u ermee akkoord gaat dat de onderzoeker gegevens over u verzamelt en dat de studiesponsor deze gegevens gebruikt voor onderzoeksdoeleinden en in verband met wetenschappelijke en medische publicaties.

U hebt het recht om de onderzoeker te vragen welke gegevens er over u verzameld worden en waarvoor ze in functie van de studie gebruikt worden. Deze gegevens omvatten uw huidige klinische situatie, maar ook wat achtergrondgegevens, de resultaten van onderzoeken uitgevoerd binnen de context van uw gezondheidszorg in overeenstemming met de huidige standaarden en natuurlijk ook de resultaten van onderzoeken vereist door het protocol. U hebt het recht om deze gegevens te inspecteren en verbeteren als ze fout zouden zijn. Deze rechten zijn gegarandeerd door de wet van 8 december 1992 op de bescherming van de persoonlijke levenssfeer in functie van het verwerken van persoonlijke gegevens en door de wet van 22 augustus 2002 over de rechten van de patiënt.

De onderzoeker is gebonden door het beroepsgeheim over wat de verzamelde gegevens betreft.

Dit betekent dat hij/zij verplicht is om nooit uw naam te vermelden in de context van een publicatie of conferentie en dat hij/zij ook uw gegevens zal coderen (uw identiteit zal vervangen worden door een ID-code in de studie) alvorens ze naar de beheerder van de database van verzamelde gegevens te versturen (Clinical Department, Promethera Biosciences).

De onderzoeker en zijn/haar team zullen daarom de enigen zijn die een link kunnen maken tussen de gegevens die tijdens de studie verzonden worden en uw medisch dossier. Voor deze studie vereist de wet dat deze link met uw dossier gedurende minstens 30 jaar en maximaal 50 jaar bewaard blijft, in overeenstemming met de Belgische wet van 19 december 2008 over het gebruik van menselijk biologisch materiaal en de van toepassing zijnde koninklijke besluiten. De persoonlijke gegevens die verstuurd worden, zullen geen combinatie van elementen bevatten waaruit u geïdentificeerd kunt worden.

Voor de beheerder van de studiegegevens die wordt aangeduid door de sponsor, zullen de verstuurde gegevens het niet mogelijk maken om u te identificeren. De beheerder is verantwoordelijk voor het verzamelen van de gegevens van alle onderzoekers die deelnemen aan deze studie en ze te verwerken en beschermen in overeenstemming met de vereisten van de Belgische wet op de bescherming van de privacy.

Om de kwaliteit van de studie te verifiëren, is het mogelijk dat uw medisch dossier onderzocht wordt door personen die gebonden zijn door het beroepsgeheim en aangewezen door het ethisch comité, de sponsor van de studie of een onafhankelijk controle-orgaan. In elk geval mag dit onderzoek van uw medisch dossier alleen plaatsvinden onder de verantwoordelijkheid van de onderzoeker en onder toezicht van één van de door hem/haar aangeduide medewerkers.

De (gecodeerde) studiegegevens zullen mogelijk verstuurd worden naar Belgische of andere regelgevende autoriteiten, de relevante ethische comités, andere artsen en/of organisaties die samenwerken met de sponsor.

Ze zullen mogelijk ook verstuurd worden naar andere vestigingen van de sponsor in België en in andere landen waar de standaarden voor het beschermen van persoonlijke gegevens mogelijk anders of minder streng zijn. Zoals hierboven uitgelegd, worden de verstuurde gegevens gecodeerd. De sponsor verbindt zich ertoe de beperkingen van de Europese Richtlijn en de Belgische wetgeving op de bescherming van de privacy te respecteren.

Uw toestemming om deel te nemen aan deze studie impliceert hierdoor uw toestemming voor het gebruik van uw gecodeerde medische gegevens voor het doel beschreven in dit informatieformulier en voor de overdracht ervan naar de hierboven vermelde personen en autoriteiten.

De sponsor verbindt zich ertoe om de gegevens alleen te gebruiken binnen de context van de studie waaraan u deelneemt.

Verzekering

Elke deelname aan een klinische studie houdt een risico in, hoe klein ook. Zelfs zonder fout aanvaardt de sponsor verantwoordelijkheid voor schade die aan de deelnemer wordt veroorzaakt (of in het geval van overlijden, zijn/haar rechthebbenden) en die rechtstreeks of onrechtstreeks verbonden is met zijn/haar deelname aan de studie. De sponsor heeft een verzekering afgesloten voor deze verantwoordelijkheid.

We vragen u daarom om elk nieuwe gezondheidsprobleem te melden aan de onderzoeker voordat u een andere arts raadpleegt, andere medicatie begint in te nemen of een andere medische behandeling ondergaat. Als u, om eender welke reden, een andere arts raadpleegt tijdens deze klinische studie, moet u hem/haar inlichten dat u deelneemt aan een klinische studie/uw deelnemerskaart van de klinische studie tonen. Dit kan belangrijk zijn voor het stellen van een diagnose en het behandelen van uw klachten.

Als de onderzoeker denkt dat een verband met de studie mogelijk is (de verzekering dekt het natuurlijke verloop van uw ziekte of de bekende bijwerkingen van uw normale behandeling niet), zal hij/zij de studiesponsor informeren, die de aangifteprocedure bij de verzekeringsmaatschappij zal opstarten. De laatste zal een deskundige aanstellen (als dit nodig wordt geacht) om te beoordelen of er een verband bestaat tussen uw nieuwe gezondheidsproblemen en de studie.

In geval van onenigheid met de onderzoeker of met de door de verzekeringsmaatschappij aangestelde deskundige en ook wanneer u dat nodig acht, kunt u, of kunnen uw nabestaanden in geval van overlijden, de verzekeraar rechtstreeks in België dagvaarden (naam van verzekeringsmaatschappij, polisnummer, contactpersoon).

De wet voorziet dat de verzekeraar voor de rechtbank moet verschijnen van de locatie waar het schadegeval optrad, voor de rechtbank van uw woonplaats, of voor de rechtbank van de hoofdzetel van de verzekeraar.

Indien u aan deze klinische studie deelneemt, verzoeken wij om:

- Ten minste 5 weken na het begin van uw deelname geen alcohol te drinken.
- Volledig mee te werken aan een rimpelloos verloop van de studie.
- Geen enkele informatie in verband met uw gezondheidstoestand, de medicatie die u neemt of de symptomen die u ondervindt, te verbergen.
- Niet deel te nemen aan een andere klinische studie waarbij er sprake is van een behandeling in het kader van het onderzoek, of het nu gaat om een medisch hulpmiddel of een procedure, terwijl men aan deze studie deelneemt.
- De "noodkaart" steeds bij u te hebben. Dit is absoluut noodzakelijk voor uw veiligheid in geval u dringende zorg nodig hebt in een instelling die u niet kent.

Contact

Indien u verder informatie nodig hebt, maar ook indien u problemen of zorgen heeft, kunt u de onderzoeker …….of een lid van zijn/haar research team..... op het volgende telefoonnummer (xx / xxx-xx-yy).....contacteren.

In noodgeval of buiten de consultatie-uren, contacteer de spoedgevallen …… op het volgend telefoonnummer …….. en vermeld dat u aan een klinische studie

deelneemt. Uw dossier zal informatie bevatten die nuttig is voor de dienstdoende arts in verband met deze klinische studie.

HEP101 STUDIE

Multicentrische klinische fase-2-studie naar de veiligheid en voorstudie naar de werkzaamheid van twee doseringsregimes van HepaStem bij patiënten met acuut leverfalen bij een chronische leverziekte

Toestemming van de geïnformeerde patiënt

ID van de patiënt in de studie: ……..

Deelnemer

Ik verklaar dat ik ingelicht ben over de aard van de studie, haar doelstelling, duur, risico's en baten, alsook over wat van mij wordt verwacht. Ik heb kennis genomen van het informatiedocument en ik heb voldoende tijd gehad om erover na te denken en te bespreken met een persoon van mijn keuze, zoals mijn huisarts of een familielid.

Ik heb de gelegenheid gehad om aan de onderzoeker de vragen te stellen die bij me opkwamen en ik heb een bevredigend antwoord op mijn vragen gekregen.

Ik begrijp dat mijn deelname aan deze studie vrijwillig is en dat ik vrij ben om mijn deelname aan deze studie te beëindigen zonder dat mijn relatie met het therapeutisch team dat verantwoordelijk is voor mijn gezondheid, in het gedrang komt.

Ik ben er me van bewust dat voor deze klinische studie mijn persoonlijke gegevens, vooral mijn medische gegevens worden verzameld, opgeslagen en geanalyseerd. Het gebruik van de informatie in verband met mijn gezondheid stemt overeen met de wettelijke bepalingen en vereist, voorafgaand aan deze studie een vrijwillig gegeven geïnformeerde toestemming. Zonder daaropvolgende toestemming kan ik niet aan de studie deelnemen.

De persoonlijke gegevens zijn geëncrypteerd. Ik heb het recht mijn gegevens te bekijken/te verbeteren.

De toestemming om mijn persoonlijke gegevens te verzamelen en te verwerken, vooral informatie over mijn gezondheid, is onherroeppelijk. Ik ben reeds geïnformeerd dat ik mijn deelname aan dit onderzoek te allen tijde kan beëindigen. Ingeval van een dergelijke terugtrekking van mijn toestemming, stem ik ermee in dat mijn gegevens die tot hiertoe zijn verzameld, onder pseudoniem (geëncrypteerd) kunnen worden geanalyseerd.

Ik stem ermee in dat mijn gegevens na beëindiging van de studie of bij onderbreking zullen worden opgeslagen volgens de nationale vereisten.

Ik stem ermee in dat de gegevens van deze studie, verzameld voor de doelstellingen van deze studie, op een later datum worden verwerkt, op voorwaarde dat de verwerking uitsluitend verband houdt met het kader van de huidige studie voor een beter begrip van de ziekte en haar behandeling.

Ik stem ermee in dat de sponsor stalen behoudt van het biologisch materiaal dat tijdens de studie, op het einde van het klinisch onderzoek voor verdere onderzoeksdoeleinden werd verzameld, maar binnen het kader van de huidige studie.

Ik stem ermee in dat mijn huisarts of andere specialisten die verantwoordelijk zijn voor mijn gezondheid, worden geïnformeerd over mijn deelname aan deze klinische studie.

Ik heb een kopie ontvangen van de informatie voor de deelnemer en een formulier voor geïnformeerde toestemming.

Achternaam van de
patiënt: ……Voornaam: ……

Handtekening: …… Datum: ……

Wettelijke vertegenwoordiger

Ik verklaar dat mij werd gevraagd om de beslissing over de eventuele deelname aan de klinische studie door de persoon die ik vertegenwoordig, te bevestigen, rekening houdend met zijn belangen en waarschijnlijke wens. Mijn instemming is van toepassing op alle punten die worden vermeld in het toestemmingsformulier van de deelnemer.

Ik heb een kopie ontvangen van het informatiedocument van de deelnemer en het formulier voor geïnformeerde toestemming.

Voornaam van de wettelijke vertegenwoordiger: ……

Achternaam van de wettelijke vertegenwoordiger:……

Handtekening: ……
Datum: ……

Getuige/Tolk

Ik was aanwezig tijdens het volledige proces van de patiënteninformatie en ik bevestig dat de informatie over de doelstellingen en de procedures van de studie naar behoren werd verleend, dat de deelnemer (of zijn/haar vertegenwoordiger) blijkbaar de studie begreep en dat de toestemming om deel te nemen in volle vrijheid werd gegeven.

De getuige/tolk: … Achternaam & Voornaam: ……..

Handtekening: …… Datum : ……

De onderzoeker

Ik, de ondergetekende,……., onderzoeker, bevestig dat ik reeds mondeling de nodige informatie over de studie heb verstrekt en dat ik aan de deelnemer een kopie van het informatief document heb gegeven.

Ik bevestig dat er geen druk werd uitgeoefend om de patiënt te overtuigen om zijn toestemming te geven om aan deze studie deel te nemen en dat ik, zo nodig, bijkomende vragen wil beantwoorden.

Ik bevestig dat ik in overeenstemming met ethische principes handel die zijn opgenomen in de laatste versie van de “Verklaring van Helsinki”, de “Goede Klinische Praktijk” en de huidige nationale regeling, in verband met onderzoeken waaraan mensen deelnemen.

Achternaam van de onderzoeker: ……Voornaam……

Handtekening: …… Datum: ……

HEP101 STUDIE

MULTICENTRISCHE KLINISCHE FASE-2-STUDIE NAAR DE VEILIGHEID EN VOORSTUDIE NAAR DE WERKZAAMHEID VAN TWEE DOSERINGSREGIMES VAN HEPASTEM BIJ PATIËNTEN MET ACUUT LEVERFALEN BIJ EEN CHRONISCHE LEVERZIEKTE.

Retrospectieve informatie

Vanwege uw klinische toestand op het moment van opname in de hierboven genoemde studie was u niet geheel in staat zelf te beslissen of u al dan niet wilde deelnemen aan deze studie.

In zulke gevallen is het gebruikelijk een wettelijke vertegenwoordiger (meestal een naast familielid) te vragen de eventuele deelname van een persoon aan de studie te bevestigen, waarbij hij/zij rekening houdt met de belangen en vermoedelijke wens van de betreffende persoon.

Het is belangrijk dat u zich realiseert dat het ethisch comité (in Leuven) na overleg met de ethische comités van andere deelnemende klinische centra heeft ingestemd met toepassing van deze procedure op …….

U bent dus opgenomen in deze studie en u hebt ingestemd met deelname, maar omdat u niet geheel in staat was zelf een beslissing te nemen, heeft de onderzoeker uw wettelijke vertegenwoordiger gevraagd uw deelname te bevestigen door eveneens dit toestemmingsformulier te ondertekenen.

Door verbetering van uw klinische toestand kunt u zich inmiddels volledig op de hoogte stellen van de doelstellingen en procedures van de studie, kunt u zelf toestemming geven en kunt u besluiten uw deelname aan deze studie voort te zetten dan wel te beëindigen.

Wij vragen u nu te bevestigen of u uw deelname wilt voortzetten of beëindigen en wij nodigen u uit dit document door te lezen. Het bevat niet alleen informatie over de doelstellingen en procedures van de studie, maar ook over de mogelijke risico's en voordelen verbonden aan het onderzoeksproduct en over uw rechten als deelnemer aan een klinische studie.

Lees de informatie zorgvuldig door. Hebt u vragen, stel deze dan aan de onderzoeker of zijn vertegenwoordiger. Dit document bestaat uit twee delen: belangrijke informatie voor het nemen van uw beslissing en een formulier voor schriftelijke toestemming.

In een dergelijke situatie zal een nieuw formulier voor geïnformeerde toestemming worden ondertekend door zowel u als door de onderzoeker van de studie.

HEP101 STUDY
MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Patient Information

This patient information and the informed consent form are for the patients or legal representatives of patients suffering from Acute-on-Chronic Liver Failure (ACLF) who are being invited to participate in a research with HepaStem

Sponsor: **Promethera Biosciences**
Address: **Rue Granbonpré 11**
1435 Mont St Guibert
Belgium

Principal Investigator: _____
Address _____
Telephone number: _____

Introduction

You are being invited to take part in a clinical study to evaluate an investigational medicinal product for the treatment of your disease. An investigational medicinal product is a medicinal product that is still being studied to evaluate its efficacy, safety or mode of action.

For the attention of the patient: It may be that when you were included in the study, you were not fully capable to decide for yourself whether or not to take part in this study. It is then customary to use a legal representative, who is asked to make a decision on the person's participation in the study in the best interests of this person and taking into consideration his/her likely wishes.

For the attention of the legal representative: Because of his/her clinical situation, we consider that the person you represent is able to decide whether or not to participate in the study, although on the occasion of his/her illness it could be questioned that they are fully aware of the implications of the study. For this reason, you are invited to decide whether the person you represent should participate in this clinical trial, taking into consideration his/her likely wishes.

In the remainder of this document, the sentences are worded as if we were directly addressing the person you represent.

The Investigator (or study doctor who is the physician responsible for this research study at the hospital) and the Sponsor (company which initiates and finances this research) expect that this medicinal product offers advantages in the treatment of patients with the same disease as yours. There is, however, no guarantee that you will benefit from taking part in this study.

Before you agree to take part in this study, we invite you to take note of its implications in terms of organisation, possible risks and benefits, to allow you to make a decision with full awareness of the implications. This is known as giving "informed consent".

Please read this information carefully and ask any questions you want to the investigator or his/her representative. There are 2 parts to this document: the information essential to your decision, and your written consent form.

Before you make your decision, you should be aware that:

- Before its start-up, this clinical study has been the subject of an evaluation by an ethics committee ([national EC to be mentioned]) after consulting the ethics committees of other participating centers. In any case, you should not consider the favorable opinion of that committee as an incitement to participate in this study.
- Your participation is voluntary and must remain free from any coercion. It requires the signature of a document expressing your consent. Even after having signed this document, you can stop taking part by informing the investigator. Your decision not to take part or to stop taking part in the study will have no impact on the quality of your care or on your relationship with the investigator.
- The data collected in the study are confidential and your anonymity is always guaranteed including during publication of the results.
- The sponsor has contracted an insurance policy in case you should suffer any damage in connection with your participation in this clinical study.

  Insurance company: [to be completed for each country]
  Policy number: [to be completed for each country]
- Your participation in this study will be totally free. No cost will be charged for the exams performed within this study. However, you will not be offered any remuneration for participating in this study.
- Expenses related to your travel to the hospital (such as your vehicule's expenses, taxis, train tickets, etc.) will be reimbursed [please precise the way of reimbursement (vouchers/payment by hospital]
- The sponsor will reimburse the hospital/investigator for all the expenses associated with the visits/consultations, the exams and the treatments specifically related to the study.
- You may contact the investigator or a member of his/her team any time should you need any additional information.

Objectives and description of the study protocol

We are inviting you to take part in a clinical study involving human hepatic cells (called progenitor cells) derived from donated livers. These cells are expected to have a combined systemic (in your whole body) and local effect in the liver. They are expected to play an immunomodulatory role, meaning that they help regulating the exacerbated inflammatory response observed in your disease and especially help resolving the current acute event. The cells being tested are called Heterologous (from another person) Human Adult Liver-derived Progenitor Cells (HHALPC) or HepaStem.
The cells are stem cells isolated from adult livers and are not embryonic stem cells. They are preserved in a specific way, and are approved by the health authorities to be used in patients in a clinical trial setting.

Twelve (12) patients will be enrolled in this study in Europe, including approximately [number of patient expected in local country] in [local country]. The investigator will propose to participate to patients, who, like you, have a diagnosed cirrhosis and Acute on Chronic Liver Failure.

The main objective of this study is to assess the safety and efficacy of multiple (2) dosages of HepaStem administered in a single or repeated doses) with an interval of minimum 1 week.

This study is an open study of various dose regimens. This means you will either receive

- a single low-dose administration of 0.25 million cells per kilogram, with a maximum of 25 million of cells per infusion (which implies the administration of a maximum 5 ml syringe); Or else,
- -a single dose of 0.50 million of cells per kilogram, with a maximum of 50 million of cells per infusion (which implies the administration of a maximum 10 ml syringe); Or else

-an administration followed by a second administration one week later at least. At each time, a dose of 0.50 million of cells per kilogram with a maximum of 50 million per infusion (which implies the administration of a maximum 10 ml syringe) will be administered.In both cases, you and your research doctor will know the dosage you are receiving.

Course of the study

After the treatment with cells and further 3 to 4 weeks of surveillance, you will remain in the study until 1 year is over (follow-up period). Within the first month you will have 8 study assessments, i.e. days with study-specific procedures and examinations. During the follow-up period, you will have 4 study assessments.

The examinations or procedures that are required in connection with the study are described below. Some of the examinations and procedures are part of the standard care provided in your hospital, while others are done specifically for the study.

Your participation in the study will include 3 Period:

1 Screening Period: during which, your investigator will verify that you are eligible to participate in this study and will collect your medical history and the data of some of the examinations that have been performed after you agreed to participate in the study.

A maximum of 32 mL (approximately 3 tablespoons of blood) will be taken. Blood samples are already done as part of the care of your current clinical situation; therefore, no additional puncture on your arm will be done to collect these blood tubes.
Some analysis of your urine will be also done.
The blood and urine samples will allow us to analyse parameters that will inform us on the status of your organs (liver, kidney, blood), on your virology status (including Hepatitis and HIV), and on your immunity.
If it has not been already done, you will have an echography of your liver and your heart, a chest X ray and an electrocardiogram. These examinations are not invasive and won't be painful.

You will receive an "emergency card" with all information regarding the study. Please keep this card with you any time. If you're hospitalized, please show this card to your doctor and inform him/her that you are participating in this study

After the screening period, if all criteria to enter the study are met you will be able to start the active study period and receive the HepaStem.

2 Active Period: During this period, you will be given 1 or 2 doses of HepaStem. Depending on the group you belong to, you will be given 0.25 million cells per kilogram (with a maximum of 25 million cells per infusion in case your weight is above 100 kilogram) or 0.5 million cells per kilogram (with a maximum of 50 million cells per infusion in case your weight is above 100 kilogram). This corresponds to a volume of 5 or 10 mL liquid.HepaStem will be administered in an intravenous way, which means

that a needle will be put in a small or large vein to administer the product. Medication will be given before HepaStem infusion to prevent adverse reactions of the body to the product.

During each administration and in any case 4, 8 and 12 days after and 2, 3 and 4 weeks after your 1st administration, you will have clinical examinations and blood samples will be taken (maximum of 25 mL, approximately 2 tablespoons of blood). The blood samples will allow to gain information on the status of your organs (liver, kidney, blood).
During each HepaStem administration there will be blood samples 4h, 8h, 12h, 24 hours, 48h and 72 hours after administration (each of these samples is a maximum of 11 ml of blood (a 1 tablespoon). These samples will allow to obtain information regarding your blood clotting function.
An echography of your heart will be performed after the 1st administration and an echography of your liver will be done before each infusion, 2 and 4 weeks after the 1st administration.

During the administration of HepaStem, a representative of the sponsor may be present to provide additional information and advice to your study doctor, who remains responsible for the administration. This representative is obliged to follow all rules of confidentiality and protection of the privacy stipulated below.

3 Follow up period: during this period, we will ask you to come back to the hospital 2 months, 3 months, 6 months and 12 months after the 1st cell infusion. At these visits, you will have a clinical examination, and a blood sample (maximum of 15 mL, approximately 1 tablespoons of blood). The results of these examinations will be collected.
The blood samples will allow us to analyse parameters that will inform us on the status of your organs (liver, kidney, blood).
During this period, if you have a liver transplantation, if possible, a sample of your explanted liver will be collected.

After the follow up period, we will ask you if you agree to be a part of our registry for additional and regular follow up.

Risks and discomforts

A: Side effects of the study medicinal product :

All medicinal products have known or unforeseeable side effects. Even if previous studies have shown that HepaStem was well tolerated, you may still experience the following side effects: **at short-term:**

- thrombosis: This means formation of a blood clot inside a blood vessel obstructing the flow in this vessel.

- bleeding: in this study, in 3 patients previously treated, two very serious cases of bleeding were observed. Therefore, careful control of your blood clotting parameters will be performed to avoid that.

- respiratory difficulties may occur

- hypersensitivity reaction or reaction to the infusion: This occurs when the body's immune system over reacts to something like medication. Hypersensitivity reactions may include: skin irritation, redness, itching, swelling, fluid discharge, crusting, skin rash, eruptions, coughing or shortness of breath, hoarse voice, headache, clogged or runny nose, sneezing, red (bloodshot) eyes, stomach pain, nausea, vomiting, diarrhea, fatigue, sore throat, dizziness. These reactions may be harmful, uncomfortable or in some cases fatal (in the case of anaphylaxis).

In the medium or long term:

- distribution in different organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of cells from another person which may eventually lead to cell rejection.

Other currently unknown risks and discomforts could appear. It is therefore very important that you report quickly any new health problem to the investigator, regardless of whether or not you think it has to do with the study.

C: Risks associated with the procedures specific to the study

There are also risks/discomforts associated with the specific examinations that will be performed for the the study:

- The intravenous administration may cause
  - Pain, as for any injection.
  - Infection. Any break in the skin carries a risk of infection although intravenous insertion is an aseptic procedure.
  - Phlebitis: inflammation of a vein that may be caused by infection.
  - Infiltration: infiltration occurs when a fluid or medication administered in the vein accidentally enters the surrounding tissue rather than the vein.
  - Embolism: A blood clot or other solid mass, or an air bubble, can be delivered into the circulation through an IV and end up blocking a vessel. However, it is nearly impossible to inject air through a peripheral IV. The risk is greater with a central IV.
- The blood sampling necessary for analysis may (rarely) cause pain, bleeding, bruising or infection localised around the puncture site. Similarly, some patients may feel dizzy or even faint during the procedure.

The staff who takes the blood will use best medical care to prevent or to keep these discomforts to a minimum.

Notification of new information

It may be that during the course of a clinical study, important new information on HepaStem being investigated becomes available. You will be informed in a timely manner of any new element that might affect your decision to continue taking part in this study.

If, in the light of the new information, you decide to stop taking part in the study, your investigator will ensure that you continue to receive the best possible treatment.

Contraception, pregnancy and breast-feeding

Female participant**:** Because the effects of HepaStem on an unborn child or infant are not properly known, you will not be allowed to take part in this clinical study if you are pregnant, wish to become pregnant or if you are breast-feeding.

If you choose to take part in this study, you must use one of the authorised methods of contraception (so that you do not become pregnant). Your doctor will discuss the various appropriate options with you.

Benefits

If you agree to take part in this study, HepaStem may or may not prove beneficial in treating your disease or relieving your symptoms, especially helping to resolve the current acute decompensation of liver function.

The information obtained with this study may contribute to a better knowledge of the use of this medicinal product or to the development of a new medicinal product for the treatment of Acute on Chronic Liver Failure in future patients.

Alternative treatment:

No treatment for your condition that has been approved by the authorities is currently available in Europe.

The only treatment currently available is liver transplantation. However, this treatment is far from ideal due to the shortage of organs for transplantation

Withdrawal from the study

Your participation is voluntary and you are entitled to withdraw from the study at any time without giving reason. Nevertheless, it may be useful for the investigator and for the sponsor of the study to know if you are withdrawing because the constraints of the treatment are too great (too many uncomfortable side effects, for example).

It is also possible that the investigator withdraws you from the study because you are pregnant, because he/she thinks it is better for your health or because he/she finds out that you are not following the instructions given to participants.

Finally, the competent national or international authorities, the ethics committee that initially approved the study or the sponsor may decide to stop the study.

If you withdraw your consent to participate in the study, in order to guarantee the validity of the investigation, the encrypted data will be retained until the point you withdrew. No new data may be sent to the sponsor.

If you withdraw your consent to participate in the study, you can contact the researcher and ask to have the samples not yet used to be destroyed. The results obtained from your samples before you withdrew your consent, remain the property of the study sponsor.

Treatment after stopping the study

In all these situations of withdrawing from the study, but also when the scheduled participation period has ended, your investigator will assess your state of health and prescribe the best treatment available.

Samples of biological material collected during the study

The sponsor of the study assures that the samples (e.g. blood, urine, liver tissue) will only be used within the context of the study.

- The procedure for encrypting samples is the same as the one used for your medical data. Therefore, samples sent to the sponsor will only contain your study ID code.

-The ones who manage these samples (the haematology Laboratory of the Cliniques St. Luc in Brussels, Belgium and the translational research Center, KU Leuven, Leuven, Belgium) will use them within the context of clinical research and destroy them at the end of the planned Retention period.

-The taken sample of biological material is considered a ' donation ' and you should be aware that, in principle, you will not receive any financial benefit (royalties) associated with the development of new therapies derived of the use of your donation of biological material, and which may have commercial value.

The surplus of your samples will be destroyed once the analyses described in this document have been carried out (at the latest, one year after the end of the study)

Confidentiality and data protection

Your participation in the study means that you agree that the researcher collects data about you and that the study sponsor uses this data for research purposes and for scientific and medical publications.

You have the right to ask the researcher what information is collected about you and for what they are used in function of the study. These data include your current clinical situation, but also some background data, the results of surveys conducted within the context of your health care in accordance with current standards and of course the results of evaluations required by the Protocol. You have the right to review and correct this data if they are wrong. These rights are guaranteed by the [local country law about data protection to be mentioned here]

The researcher is bound by the professional secrecy with regard to the data collected.

This means that he/she is obliged to never disclose your name in the context of a publication or conference and that he/she will also encrypt your data (your identity will be replaced by an ID code in the study) before sending collected data to the database administrator (Clinical Department of Promethera Bioscience).

The researcher and his/her team will therefore be the only ones who can link the data sent during the study and your medical dossier. For this study, the law requires that this link is retained with your dossier for at least 30 years and for a maximum of 50 years, in accordance with [local country law to be mentioned here]

The personal information that is sent will not contain any combination of elements from which you can be identified.

For the administrator of the study data that is indicated by the sponsor, the data sent will not allow you to identify. The Administrator is responsible for collecting the data of all researchers who participate in this study and to process and protect them in accordance with the requirements of the Belgian Law on the protection of privacy.

In order to verify the quality of the study, it is possible that your medical dossier is examined by persons bound by the professional secrecy and designated by the Ethics Committee, the sponsor of the study or an independent supervisory body. In any case, this examination of your medical dossier may only take place under the responsibility of the researcher and supervised by one of the employees appointed by him/her.

The (coded) study data may be sent to national regulatory authorities, the relevant ethics committees, other doctors and/or organisations that collaborate with the sponsor.

They may also be sent to other branches of the sponsor in Belgium and other countries where the standards for protecting personal data may be different or less stringent. As explained above, the data sent is encrypted. The sponsor undertakes to respect the limitations of the European directive and the Belgian legislation on the protection of privacy.

Your consent to participate in this study implies your consent to the use of your coded medical data for the purpose described in this information form and for its transfer to the above-mentioned persons and Authorities. The sponsor agrees to use the data only in the context of the study in which you participate.

Insurance:

Any participation in a clinical study involves a risk, however small. The sponsor is, even without fault, liable for the damage which the subject and his rightful claimants (in case of death) sustained and which shows either a direct or an indirect connection with the trial. An insurance covering this liability has been established. You are

therefore asked to report the investigator of any new health problems before consulting another doctor, taking another medication or receiving any other medical treatment. If for any reason you consult another physician during this clinical trial, you must inform them that you are participating in a clinical trial/present them with your clinical trial participant card. This can be important for diagnosing and handling your complaints.

If the investigator believes a link to the study is possible (the insurance does not cover the natural progression of your illness or the known side effects of your normal treatment), he/she will inform the Sponsor who will initiate the reporting procedure to the insurance company. They will appoint an expert - if they consider it necessary - to determine if there is a link between your new health problem and the study.

In the event of a disagreement with the investigator or expert appointed by the insurance company, as well as whenever you consider it useful, you or - in the event of death - your beneficiaries can initiate proceedings against the insurer directly in [Country] [name of the insurance company, policy number, contact person]. The law ensures that the insurer can be summoned by the judge of the place where the incident giving rise to the damage occurred, or before the judge of your domicile, or before the judge of the head office of the insurer.

If you take part in this clinical study, we ask you:

- To not drink alcohol for at least 5 weeks from the start of your participation.
- To cooperate fully in the smooth running of this study.
- Not to conceal any information relating to your state of health, the medication you are taking or the symptoms you are experiencing.
- Not to take part in other clinical study involving an investigational treatment, be it a medicinal product, a medical device or a procedure, while taking part in this study.
- To carry the "emergency card" with you at all times. This is imperative for your safety in the event of emergency care in an institution that does not know you.

Contact

If you need further information, but also if you have problems or concerns, you can contact the investigator ……..or a member of his/her research team.....on the following telephone number (xx / xxx-xx-yy) ……..

In case of emergency or outside consulting hours, you can contact the emergency department…… on the following telephone number.....indicating that you are taking part in a clinical study. Your records will contain information of use to the on-call doctor in relation to this clinical study.

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HEP101 STUDY

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Patient Informed Consent

Study Patient ID : ……..

Participant

I declare that I have been informed of the nature of the study, its purpose, its duration, any risks and benefits and what is expected of me. I have taken note of the information document I have had sufficient time to think about it and discuss it with a person of my choice, such as my general practitioner (GP) or a member of my family.

I have had the opportunity to ask the investigator any questions that came to mind and have obtained a satisfactory response to my questions.

I understand that my participation in this study is voluntary and that I am free to end my participation in this study without this affecting my relationship with the therapeutic team in charge of my health.

I am aware that for this clinical study, personal data (especially my medical records) be collected, stored and analysed. The use of the information concerning my health is in accordance with legal provisions and requires a voluntarily given informed consent prior to my participation in this study. Without the subsequent agreement I cannot participate in the study.
Personal data is encrypted. I have the right to review/correct my data.

The consent to the collection and processing of my personal data, especially information about my health is irrevocable. I have already been informed that I can stop participating in this trial at any time. In case of such withdrawal of my consent, I agree that data collected until this point can be analyzed pseudonymously (encrypted).

I agree that my data will be kept after the end of the study or discontinuation of the study, in accordance with the required national requirements.

I agree to the study data collected in order to achieve the objectives of this study may be further processed, provided that such treatment is limited to the context of the present study for a better understanding of the disease and its treatment.

I agree to the sponsor retaining samples of biological material collected during the course of the study after the completion of the clinical study for subsequent research purposes, but limited to the context of this study.

I agree my GP or other specialists in charge of my health, being informed of my participation in this clinical study.

I have received a copy of the information to the participant and the informed consent form.

Patient's First Name: ……Name: ……

Signature: …… Date: ……

Legal representative

I declare that I am being asked to take a decision on whether or not to take part in the clinical study for the person I represent in his/her best interests and taking into consideration his/her likely wishes. My consent applies to all the items listed in the consent of the participant.

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I have received a copy of the information to the participant and the informed consent form.

legal representative's First Name: ……Name ……

Signature: …… Date: ……

Witness/Interpreter

I was present during the entire process of informing the patient and I confirm that the information on the objectives and procedures of the study was adequately provided, that the participant (or his/her legal representative) apparently understood the study and that consent to participate in the study was freely given.

the witness/interpreter: … First Name & Name: ……..

Signature: …… Date : ……

Investigator

I, the undersigned,……., investigator, confirm that I have verbally provided the necessary information about the study and have given the participant a copy of the information document.

I confirm that no pressure was applied to persuade the patient to agree to take part in the study and that I am willing to answer any additional questions if required.

I confirm that I operate in accordance with the ethical principles set out in the latest version of the "Helsinki Declaration", the "Good Clinical Practices" and current national regulation related to trials that involve the participation of human subjects.

First Name of the investigator: ……Name……

Signature: …… Date : ……

HEP101 STUDY

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Retrospective Information

Given your clinical condition when you were admitted to intensive care, you were unable to decide for yourself whether or not to take part in the above-mentioned study.

It is then customary to use a legal representative (usually a close relative), who is asked to make a decision on the person's participation in the study in the best interests of this person and taking into consideration his/her likely wishes.

You should be aware that the ethics committee agreed to the application to this study of the emergency procedure on .....

You were therefore included in this study, you agreed to participate, but, not being fully capable of deciding for yourself, the investigator asked your legal representative to confirm your participation by signing the consent.

Now, improving your clinical situation allows you to be properly informed about the objectives and procedures of the study and give your consent to continue or discontinue your participation in the study.

We are now asking you to confirm your wish to continue or discontinue this participation, and we invite you to take note of the document informing you fully on the objectives and procedures of the study, as well as on the possible risks and benefits of the investigational treatment and your rights as a participant in a clinical study.

Please read these few pages of information carefully and ask any questions you want to the investigator or his/her representative. There are 2 parts to this document: the information essential to your decision and your written consent.

In this case, a new informed consent will be re-signed by you and the investigator of the study.

HEP101 STUDY
MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Patient Information

This patient information and the informed consent form are for the patients or legal representatives of patients suffering from Acute-on-Chronic Liver Failure (ACLF) who are being invited to participate in a research with HepaStem

Sponsor: **Promethera Biosciences**
Address: **Rue Granbonpré 11**
1435 Mont St Guibert
Belgium

Principal Investigator: _____
Address _____
Telephone number: _____

Introduction

You are being invited to take part in a clinical study to evaluate an investigational medicinal product for the treatment of your disease. An investigational medicinal product is a medicinal product that is still being studied to evaluate its efficacy, safety or mode of action.

For the attention of the patient: It may be that when you were included in the study, you were not fully capable to decide for yourself whether or not to take part in this study. It is then customary to use a legal representative, who is asked to make a decision on the person's participation in the study in the best interests of this person and taking into consideration his/her likely wishes.

For the attention of the legal representative: Because of his/her clinical situation, we consider that the person you represent is able to decide whether or not to participate in the study, although on the occasion of his/her illness it could be questioned that they are fully aware of the implications of the study. For this reason, you are invited to decide whether the person you represent should participate in this clinical trial, taking into consideration his/her likely wishes.

In the remainder of this document, the sentences are worded as if we were directly addressing the person you represent.

The Investigator (or study doctor who is the physician responsible for this research study at the hospital) and the Sponsor (company which initiates and finances this research) expect that this medicinal product offers advantages in the treatment of patients with the same disease as yours. There is, however, no guarantee that you will benefit from taking part in this study.

Before you agree to take part in this study, we invite you to take note of its implications in terms of organisation, possible risks and benefits, to allow you to make a decision with full awareness of the implications. This is known as giving "informed consent".

Please read this information carefully and ask any questions you want to the investigator or his/her representative. There are 2 parts to this document: the information essential to your decision, and your written consent form.

Before you make your decision, you should be aware that:

- Before its start-up, this clinical study has been the subject of an evaluation by an ethics committee ([national EC to be mentioned]) after consulting the ethics committees of other participating centers. In any case, you should not consider the favorable opinion of that committee as an incitement to participate in this study.
- Your participation is voluntary and must remain free from any coercion. It requires the signature of a document expressing your consent. Even after having signed this document, you can stop taking part by informing the investigator. Your decision not to take part or to stop taking part in the study will have no impact on the quality of your care or on your relationship with the investigator.
- The data collected in the study are confidential and your anonymity is always guaranteed including during publication of the results.
- The sponsor has contracted an insurance policy in case you should suffer any damage in connection with your participation in this clinical study.

  Insurance company: [to be completed for each country]
  Policy number: [to be completed for each country]
- Your participation in this study will be totally free. No cost will be charged for the exams performed within this study. However, you will not be offered any remuneration for participating in this study.
- Expenses related to your travel to the hospital (such as your vehicule's expenses, taxis, train tickets, etc.) will be reimbursed [please precise the way of reimbursement (vouchers/payment by hospital]
- The sponsor will reimburse the hospital/investigator for all the expenses associated with the visits/consultations, the exams and the treatments specifically related to the study.
- You may contact the investigator or a member of his/her team any time should you need any additional information.

Objectives and description of the study protocol

We are inviting you to take part in a clinical study involving human hepatic cells (called progenitor cells) derived from donated livers. These cells are expected to have a combined systemic (in your whole body) and local effect in the liver. They are expected to play an immunomodulatory role, meaning that they help regulating the exacerbated inflammatory response observed in your disease and especially help resolving the current acute event. The cells being tested are called Heterologous (from another person) Human Adult Liver-derived Progenitor Cells (HHALPC) or HepaStem.
The cells are stem cells isolated from adult livers and are not embryonic stem cells. They are preserved in a specific way, and are approved by the health authorities to be used in patients in a clinical trial setting.

Twelve (12) patients will be enrolled in this study in Europe, including approximately [number of patient expected in local country] in [local country]. The investigator will propose to participate to patients, who, like you, have a diagnosed cirrhosis and Acute on Chronic Liver Failure.

The main objective of this study is to assess the safety and efficacy of multiple (2) dosages of HepaStem administered in a single or repeated doses) with an interval of minimum 1 week.

This study is an open study of various dose regimens. This means you will either receive

- a single low-dose administration of 0.25 million cells per kilogram, with a maximum of 25 million of cells per infusion (which implies the administration of a maximum 5 ml syringe); Or else,
- -a single dose of 0.50 million of cells per kilogram, with a maximum of 50 million of cells per infusion (which implies the administration of a maximum 10 ml syringe); Or else

-an administration followed by a second administration one week later at least. At each time, a dose of 0.50 million of cells per kilogram with a maximum of 50 million per infusion (which implies the administration of a maximum 10 ml syringe) will be administered. In both cases, you and your research doctor will know the dosage you are receiving.

Course of the study

After the treatment with cells and further 3 to 4 weeks of surveillance, you will remain in the study until 1 year is over (follow-up period). Within the first month you will have 8 study assessments, i.e. days with study-specific procedures and examinations. During the follow-up period, you will have 4 study assessments.

The examinations or procedures that are required in connection with the study are described below. Some of the examinations and procedures are part of the standard care provided in your hospital, while others are done specifically for the study.

Your participation in the study will include 3 Period:

1 Screening Period: during which, your investigator will verify that you are eligible to participate in this study and will collect your medical history and the data of some of the examinations that have been performed after you agreed to participate in the study.

A maximum of 32 mL (approximately 3 tablespoons of blood) will be taken. Blood samples are already done as part of the care of your current clinical situation; therefore, no additional puncture on your arm will be done to collect these blood tubes.
Some analysis of your urine will be also done.
The blood and urine samples will allow us to analyse parameters that will inform us on the status of your organs (liver, kidney, blood), on your virology status (including Hepatitis and HIV), and on your immunity.
If it has not been already done, you will have an echography of your liver and your heart, a chest X ray and an electrocardiogram. These examinations are not invasive and won't be painful.

You will receive an "emergency card" with all information regarding the study. Please keep this card with you any time. If you're hospitalized, please show this card to your doctor and inform him/her that you are participating in this study

After the screening period, if all criteria to enter the study are met you will be able to start the active study period and receive the HepaStem.

2 Active Period: During this period, you will be given 1 or 2 doses of HepaStem. Depending on the group you belong to, you will be given 0.25 million cells per kilogram (with a maximum of 25 million cells per infusion in case your weight is above 100 kilogram) or 0.5 million cells per kilogram (with a maximum of 50 million cells per infusion in case your weight is above 100 kilogram). This corresponds to a volume of 5 or 10 mL liquid.HepaStem will be administered in an intravenous way, which means

that a needle will be put in a small or large vein to administer the product. Medication will be given before HepaStem infusion to prevent adverse reactions of the body to the product.

During each administration and in any case 4, 8 and 12 days after and 2, 3 and 4 weeks after your 1st administration, you will have clinical examinations and blood samples will be taken (maximum of 25 mL, approximately 2 tablespoons of blood). The blood samples will allow to gain information on the status of your organs (liver, kidney, blood).
During each HepaStem administration there will be blood samples 4h, 8h, 12h, 24 hours, 48h and 72 hours after administration (each of these samples is a maximum of 11 ml of blood (a 1 tablespoon). These samples will allow to obtain information regarding your blood clotting function.
An echography of your heart will be performed after the 1st administration and an echography of your liver will be done before each infusion, 2 and 4 weeks after the 1st administration.

During the administration of HepaStem, a representative of the sponsor may be present to provide additional information and advice to your study doctor, who remains responsible for the administration. This representative is obliged to follow all rules of confidentiality and protection of the privacy stipulated below.

3 Follow up period: during this period, we will ask you to come back to the hospital 2 months, 3 months, 6 months and 12 months after the 1st cell infusion. At these visits, you will have a clinical examination, and a blood sample (maximum of 15 mL, approximately 1 tablespoons of blood). The results of these examinations will be collected.
The blood samples will allow us to analyse parameters that will inform us on the status of your organs (liver, kidney, blood).
During this period, if you have a liver transplantation, if possible, a sample of your explanted liver will be collected.

After the follow up period, we will ask you if you agree to be a part of our registry for additional and regular follow up.

Risks and discomforts

A: Side effects of the study medicinal product :

All medicinal products have known or unforeseeable side effects. Even if previous studies have shown that HepaStem was well tolerated, you may still experience the following side effects: **at short-term:**

- thrombosis: This means formation of a blood clot inside a blood vessel obstructing the flow in this vessel.

- bleeding: in this study, among patients previously treated, two very serious cases of bleeding were observed. Therefore, careful control of your blood clotting parameters will be performed to avoid that.

- respiratory difficulties may occur

- hypersensitivity reaction or reaction to the infusion: This occurs when the body's immune system over reacts to something like medication. Hypersensitivity reactions may include: skin irritation, redness, itching, swelling, fluid discharge, crusting, skin rash, eruptions, coughing or shortness of breath, hoarse voice, headache, clogged or runny nose, sneezing, red (bloodshot) eyes, stomach pain, nausea, vomiting, diarrhea, fatigue, sore throat, dizziness. These reactions may be harmful, uncomfortable or in some cases fatal (in the case of anaphylaxis).

In the medium or long term:

- distribution in different organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of cells from another person which may eventually lead to cell rejection.

Other currently unknown risks and discomforts could appear. It is therefore very important that you report quickly any new health problem to the investigator, regardless of whether or not you think it has to do with the study.

C: Risks associated with the procedures specific to the study

There are also risks/discomforts associated with the specific examinations that will be performed for the study:

- The intravenous administration may cause
  - Pain, as for any injection.
  - Infection. Any break in the skin carries a risk of infection although intravenous insertion is an aseptic procedure.
  - Phlebitis: inflammation of a vein that may be caused by infection.
  - Infiltration: infiltration occurs when a fluid or medication administered in the vein accidentally enters the surrounding tissue rather than the vein.
  - Embolism: A blood clot or other solid mass, or an air bubble, can be delivered into the circulation through an IV and end up blocking a vessel. However, it is nearly impossible to inject air through a peripheral IV. The risk is greater with a central IV.
- The blood sampling necessary for analysis may (rarely) cause pain, bleeding, bruising or infection localised around the puncture site. Similarly, some patients may feel dizzy or even faint during the procedure.

The staff who takes the blood will use best medical care to prevent or to keep these discomforts to a minimum.

Notification of new information

It may be that during the course of a clinical study, important new information on HepaStem being investigated becomes available. You will be informed in a timely manner of any new element that might affect your decision to continue taking part in this study.

If, in the light of the new information, you decide to stop taking part in the study, your investigator will ensure that you continue to receive the best possible treatment.

Contraception, pregnancy and breast-feeding

Female participant**:** Because the effects of HepaStem on an unborn child or infant are not properly known, you will not be allowed to take part in this clinical study if you are pregnant, wish to become pregnant or if you are breast-feeding.

If you choose to take part in this study, you must use one of the authorised methods of contraception (so that you do not become pregnant). Your doctor will discuss the various appropriate options with you.

Benefits

If you agree to take part in this study, HepaStem may or may not prove beneficial in treating your disease or relieving your symptoms, especially helping to resolve the current acute decompensation of liver function.

The information obtained with this study may contribute to a better knowledge of the use of this medicinal product or to the development of a new medicinal product for the treatment of Acute on Chronic Liver Failure in future patients.

Alternative treatment:

No treatment for your condition that has been approved by the authorities is currently available in Europe.

The only treatment currently available is liver transplantation. However, this treatment is far from ideal due to the shortage of organs for transplantation

Withdrawal from the study

Your participation is voluntary and you are entitled to withdraw from the study at any time without giving reason. Nevertheless, it may be useful for the investigator and for the sponsor of the study to know if you are withdrawing because the constraints of the treatment are too great (too many uncomfortable side effects, for example).

It is also possible that the investigator withdraws you from the study because you are pregnant, because he/she thinks it is better for your health or because he/she finds out that you are not following the instructions given to participants.

Finally, the competent national or international authorities, the ethics committee that initially approved the study or the sponsor may decide to stop the study.

If you withdraw your consent to participate in the study, in order to guarantee the validity of the investigation, the encrypted data will be retained until the point you withdrew. No new data may be sent to the sponsor.

If you withdraw your consent to participate in the study, you can contact the researcher and ask to have the samples not yet used to be destroyed. The results obtained from your samples before you withdrew your consent, remain the property of the study sponsor.

Treatment after stopping the study

In all these situations of withdrawing from the study, but also when the scheduled participation period has ended, your investigator will assess your state of health and prescribe the best treatment available.

Samples of biological material collected during the study

The sponsor of the study assures that the samples (e.g. blood, urine, liver tissue) will only be used within the context of the study.

- The procedure for encrypting samples is the same as the one used for your medical data. Therefore, samples sent to the sponsor will only contain your study ID code.

-The ones who manage these samples (the haematology Laboratory of the Cliniques St. Luc in Brussels, Belgium and the translational research Center, KU Leuven, Leuven, Belgium) will use them within the context of clinical research and destroy them at the end of the planned Retention period.

-The taken sample of biological material is considered a ' donation ' and you should be aware that, in principle, you will not receive any financial benefit (royalties) associated with the development of new therapies derived of the use of your donation of biological material, and which may have commercial value.

The surplus of your samples will be destroyed once the analyses described in this document have been carried out (at the latest, one year after the end of the study)

Confidentiality and data protection

Your participation in the study means that you agree that the researcher collects data about you and that the study sponsor uses this data for research purposes and for scientific and medical publications.

You have the right to ask the researcher what information is collected about you and for what they are used in function of the study. These data include your current clinical situation, but also some background data, the results of surveys conducted within the context of your health care in accordance with current standards and of course the results of evaluations required by the Protocol. You have the right to review and correct this data if they are wrong. These rights are guaranteed by the [local country law about data protection to be mentioned here]

The researcher is bound by the professional secrecy with regard to the data collected.

This means that he/she is obliged to never disclose your name in the context of a publication or conference and that he/she will also encrypt your data (your identity will be replaced by an ID code in the study) before sending collected data to the database administrator (Clinical Department of Promethera Bioscience).

The researcher and his/her team will therefore be the only ones who can link the data sent during the study and your medical dossier. For this study, the law requires that this link is retained with your dossier for at least 30 years and for a maximum of 50 years, in accordance with [local country law to be mentioned here]

The personal information that is sent will not contain any combination of elements from which you can be identified.

For the administrator of the study data that is indicated by the sponsor, the data sent will not allow you to identify. The Administrator is responsible for collecting the data of all researchers who participate in this study and to process and protect them in accordance with the requirements of the Belgian Law on the protection of privacy.

In order to verify the quality of the study, it is possible that your medical dossier is examined by persons bound by the professional secrecy and designated by the Ethics Committee, the sponsor of the study or an independent supervisory body. In any case, this examination of your medical dossier may only take place under the responsibility of the researcher and supervised by one of the employees appointed by him/her.

The (coded) study data may be sent to national regulatory authorities, the relevant ethics committees, other doctors and/or organisations that collaborate with the sponsor.

They may also be sent to other branches of the sponsor in Belgium and other countries where the standards for protecting personal data may be different or less stringent. As explained above, the data sent is encrypted. The sponsor undertakes to respect the limitations of the European directive and the Belgian legislation on the protection of privacy.

Your consent to participate in this study implies your consent to the use of your coded medical data for the purpose described in this information form and for its transfer to the above-mentioned persons and Authorities. The sponsor agrees to use the data only in the context of the study in which you participate.

Insurance:

Any participation in a clinical study involves a risk, however small. The sponsor is, even without fault, liable for the damage which the subject and his rightful claimants (in case of death) sustained and which shows either a direct or an indirect connection with the trial. An insurance covering this liability has been established. You are

therefore asked to report the investigator of any new health problems before consulting another doctor, taking another medication or receiving any other medical treatment. If for any reason you consult another physician during this clinical trial, you must inform them that you are participating in a clinical trial/present them with your clinical trial participant card. This can be important for diagnosing and handling your complaints.

If the investigator believes a link to the study is possible (the insurance does not cover the natural progression of your illness or the known side effects of your normal treatment), he/she will inform the Sponsor who will initiate the reporting procedure to the insurance company. They will appoint an expert - if they consider it necessary - to determine if there is a link between your new health problem and the study.

In the event of a disagreement with the investigator or expert appointed by the insurance company, as well as whenever you consider it useful, you or - in the event of death - your beneficiaries can initiate proceedings against the insurer directly in [Country] [name of the insurance company, policy number, contact person]. The law ensures that the insurer can be summoned by the judge of the place where the incident giving rise to the damage occurred, or before the judge of your domicile, or before the judge of the head office of the insurer.

If you take part in this clinical study, we ask you:

- To not drink alcohol for at least 5 weeks from the start of your participation.
- To cooperate fully in the smooth running of this study.
- Not to conceal any information relating to your state of health, the medication you are taking or the symptoms you are experiencing.
- Not to take part in other clinical study involving an investigational treatment, be it a medicinal product, a medical device or a procedure, while taking part in this study.
- To carry the "emergency card" with you at all times. This is imperative for your safety in the event of emergency care in an institution that does not know you.

Contact

If you need further information, but also if you have problems or concerns, you can contact the investigator ……..or a member of his/her research team.....on the following telephone number (xx / xxx-xx-yy) ……..

In case of emergency or outside consulting hours, you can contact the emergency department…… on the following telephone number.....indicating that you are taking part in a clinical study. Your records will contain information of use to the on-call doctor in relation to this clinical study.

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HEP101 STUDY

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Patient Informed Consent

Study Patient ID : ……..

Participant

I declare that I have been informed of the nature of the study, its purpose, its duration, any risks and benefits and what is expected of me. I have taken note of the information document I have had sufficient time to think about it and discuss it with a person of my choice, such as my general practitioner (GP) or a member of my family.

I have had the opportunity to ask the investigator any questions that came to mind and have obtained a satisfactory response to my questions.

I understand that my participation in this study is voluntary and that I am free to end my participation in this study without this affecting my relationship with the therapeutic team in charge of my health.

I am aware that for this clinical study, personal data (especially my medical records) be collected, stored and analysed. The use of the information concerning my health is in accordance with legal provisions and requires a voluntarily given informed consent prior to my participation in this study. Without the subsequent agreement I cannot participate in the study.
Personal data is encrypted. I have the right to review/correct my data.

The consent to the collection and processing of my personal data, especially information about my health is irrevocable. I have already been informed that I can stop participating in this trial at any time. In case of such withdrawal of my consent, I agree that data collected until this point can be analyzed pseudonymously (encrypted).

I agree that my data will be kept after the end of the study or discontinuation of the study, in accordance with the required national requirements.

I agree to the study data collected in order to achieve the objectives of this study may be further processed, provided that such treatment is limited to the context of the present study for a better understanding of the disease and its treatment.

I agree to the sponsor retaining samples of biological material collected during the course of the study after the completion of the clinical study for subsequent research purposes, but limited to the context of this study.

I agree my GP or other specialists in charge of my health, being informed of my participation in this clinical study.

I have received a copy of the information to the participant and the informed consent form.

Patient's First Name: ……Name: ……

Signature: …… Date: ……

Legal representative

I declare that I am being asked to take a decision on whether or not to take part in the clinical study for the person I represent in his/her best interests and taking into consideration his/her likely wishes. My consent applies to all the items listed in the consent of the participant.

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I have received a copy of the information to the participant and the informed consent form.

legal representative's First Name: ……Name ……

Signature: …… Date: ……

Witness/Interpreter

I was present during the entire process of informing the patient and I confirm that the information on the objectives and procedures of the study was adequately provided, that the participant (or his/her legal representative) apparently understood the study and that consent to participate in the study was freely given.

the witness/interpreter: … First Name & Name: ……..

Signature: …… Date : ……

Investigator

I, the undersigned,……., investigator, confirm that I have verbally provided the necessary information about the study and have given the participant a copy of the information document.

I confirm that no pressure was applied to persuade the patient to agree to take part in the study and that I am willing to answer any additional questions if required.

I confirm that I operate in accordance with the ethical principles set out in the latest version of the "Helsinki Declaration", the "Good Clinical Practices" and current national regulation related to trials that involve the participation of human subjects.

First Name of the investigator: ……Name……

Signature: …… Date : ……

HEP101 STUDY

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Retrospective Information

Given your clinical condition when you were admitted to intensive care, you were unable to decide for yourself whether or not to take part in the above-mentioned study.

It is then customary to use a legal representative (usually a close relative), who is asked to make a decision on the person's participation in the study in the best interests of this person and taking into consideration his/her likely wishes.

You should be aware that the ethics committee agreed to the application to this study of the emergency procedure on .....

You were therefore included in this study, you agreed to participate, but, not being fully capable of deciding for yourself, the investigator asked your legal representative to confirm your participation by signing the consent.

Now, improving your clinical situation allows you to be properly informed about the objectives and procedures of the study and give your consent to continue or discontinue your participation in the study.

We are now asking you to confirm your wish to continue or discontinue this participation, and we invite you to take note of the document informing you fully on the objectives and procedures of the study, as well as on the possible risks and benefits of the investigational treatment and your rights as a participant in a clinical study.

Please read these few pages of information carefully and ask any questions you want to the investigator or his/her representative. There are 2 parts to this document: the information essential to your decision and your written consent.

In this case, a new informed consent will be re-signed by you and the investigator of the study.

HEP101 STUDY

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Patient Information

This patient information and the informed consent form are for the patients or legal representatives of patients suffering from Acute-on-Chronic Liver Failure (ACLF) who are being invited to participate in a research with HepaStem

Sponsor: **Promethera Biosciences**
Address: **Rue Granbonpré 11**
1435 Mont St Guibert
Belgium

Principal Investigator: _____
Address _____
Telephone number: _____

Introduction

You are being invited to take part in a clinical study to evaluate an investigational medicinal product for the treatment of your disease. An investigational medicinal product is a medicinal product that is still being studied to evaluate its efficacy, safety or mode of action.

For the attention of the patient: It may be that when you were included in the study, you were not fully capable to decide for yourself whether or not to take part in this study. It is then customary to use a legal representative, who is asked to make a decision on the person's participation in the study in the best interests of this person and taking into consideration his/her likely wishes.

For the attention of the legal representative: Because of his/her clinical situation, we consider that the person you represent is able to decide whether or not to participate in the study, although on the occasion of his/her illness it could be questioned that they are fully aware of the implications of the study. For this reason, you are invited to decide whether the person you represent should participate in this clinical trial, taking into consideration his/her likely wishes.

In the remainder of this document, the sentences are worded as if we were directly addressing the person you represent.

The Investigator (or study doctor who is the physician responsible for this research study at the hospital) and the Sponsor (company which initiates and finances this research) expect that this medicinal product offers advantages in the treatment of patients with the same disease as yours. There is, however, no guarantee that you will benefit from taking part in this study.

Before you agree to take part in this study, we invite you to take note of its implications in terms of organisation, possible risks and benefits, to allow you to make a decision with full awareness of the implications. This is known as giving "informed consent".

Please read this information carefully and ask any questions you want to the investigator or his/her representative. There are 2 parts to this document: the information essential to your decision, and your written consent form.

Before you make your decision, you should be aware that:

- Before its start-up, this clinical study has been the subject of an evaluation by an ethics committee ([national EC to be mentioned]) after consulting the ethics committees of other participating centers. In any case, you should not consider the favorable opinion of that committee as an incitement to participate in this study.
- Your participation is voluntary and must remain free from any coercion. It requires the signature of a document expressing your consent. Even after having signed this document, you can stop taking part by informing the investigator. Your decision not to take part or to stop taking part in the study will have no impact on the quality of your care or on your relationship with the investigator.
- The data collected in the study are confidential and your anonymity is always guaranteed including during publication of the results.
- The sponsor has contracted an insurance policy in case you should suffer any damage in connection with your participation in this clinical study.

  Insurance company: [to be completed for each country]
  Policy number: [to be completed for each country]
- Your participation in this study will be totally free. No cost will be charged for the exams performed within this study. However, you will not be offered any remuneration for participating in this study.
- Expenses related to your travel to the hospital (such as your vehicule's expenses, taxis, train tickets, etc.) will be reimbursed [please precise the way of reimbursement (vouchers/payment by hospital]
- The sponsor will reimburse the hospital/investigator for all the expenses associated with the visits/consultations, the exams and the treatments specifically related to the study.
- You may contact the investigator or a member of his/her team any time should you need any additional information.

Objectives and description of the study protocol

We are inviting you to take part in a clinical study involving human hepatic cells (called progenitor cells) derived from donated livers. These cells are expected to have a combined systemic (in your whole body) and local effect in the liver. They are expected to play an immunomodulatory role, meaning that they help regulating the exacerbated inflammatory response observed in your disease and especially help resolving the current acute event. The cells being tested are called Heterologous (from another person) Human Adult Liver-derived Progenitor Cells (HHALPC) or HepaStem.
The cells are stem cells isolated from adult livers and are not embryonic stem cells. They are preserved in a specific way, and are approved by the health authorities to be used in patients in a clinical trial setting.

Eighteen (18) to twenty-one (21) patients will be enrolled in this study in Europe, including approximately [number of patient expected in local country] in [local country]. The investigator will propose to participate to patients, who, like you, have a diagnosed cirrhosis and Acute on Chronic Liver Failure.

The main objective of this study is to assess the safety and efficacy of multiple (2) dosages of HepaStem administered in a single or repeated doses, with an interval of minimum 1 week.

This study is an open study of various dose regimens. This means you will either receive

- a single low-dose administration of 0.25 million cells per kilogram, with a maximum of 25 million of cells per infusion (which implies the administration of a maximum 5 ml syringe); Or else,
- a single dose of 0.50 million of cells per kilogram, with a maximum of 50 million of cells per infusion (which implies the administration of a maximum 10 ml syringe); Or else

a single dose of 1.0 million of cells per kilogram, with a maximum of 100 million of cells per infusion (which implies the administration of a maximum 20 ml syringe); Or else,an administration followed by a second administration one week later at least. The dosage of the second administration will be equal to the first administered dosage. A dose of 0.50 million of cells per kilogram with a maximum of 50 million per infusion (which implies the administration of a maximum 10 ml syringe) or a dose of 1.0 million of cells per kilogram with a maximum of 100 million per infusion (which implies the administration of a maximum 20 ml syringe)will be administered.

In all cases, you and your research doctor will know the dosage you are receiving.

Course of the study

After the treatment with cells and further 3 to 4 weeks of surveillance, you will remain in the study until 1 year is over (follow-up period). Within the first month you will have 8 study assessments, i.e. days with study-specific procedures and examinations. During the follow-up period, you will have 4 study assessments.

The examinations or procedures that are required in connection with the study are described below. Some of the examinations and procedures are part of the standard care provided in your hospital, while others are done specifically for the study.

Your participation in the study will include 3 Period:

1 Screening Period: during which, your investigator will verify that you are eligible to participate in this study and will collect your medical history and the data of some of the examinations that have been performed after you agreed to participate in the study.

A maximum of 32 mL (approximately 3 tablespoons of blood) will be taken. Blood samples are already done as part of the care of your current clinical situation; therefore, no additional puncture on your arm will be done to collect these blood tubes.
Some analysis of your urine will be also done.
The blood and urine samples will allow us to analyse parameters that will inform us on the status of your organs (liver, kidney, blood), on your virology status (including Hepatitis and HIV), and on your immunity.
If it has not been already done, you will have an echography of your liver and your heart, a chest X ray and an electrocardiogram. These examinations are not invasive and won't be painful.

You will receive an "emergency card" with all information regarding the study. Please keep this card with you any time. If you're hospitalized, please show this card to your doctor and inform him/her that you are participating in this study

After the screening period, if all criteria to enter the study are met you will be able to start the active study period and receive the HepaStem.

2 Active Period: During this period, you will be given 1 or 2 doses of HepaStem. Depending on the group you belong to, you will be given 0.25 million cells per kilogram (with a maximum of 25 million cells per infusion in case your weight is above 100 kilogram), 0.5 million cells per kilogram (with a maximum of 50 million cells per infusion in case your weight is above 100 kilogram) or 1.0 million cells per kilogram (with a maximum of 100 million cells per infusion in case your weight is above 100 kilogram). This corresponds to a volume of 5, 10 or 20 mL liquid. HepaStem will be administered in an intravenous way, which means that a needle will be put in a small or large vein to administer the product. Medication will be given before HepaStem infusion to prevent adverse reactions of the body to the product.

During each administration and in any case 4, 8 and 12 days after and 2, 3 and 4 weeks after your 1st administration, you will have clinical examinations and blood samples will be taken (maximum of 25 mL, approximately 2 tablespoons of blood). The blood samples will allow to gain information on the status of your organs (liver, kidney, blood).
During each HepaStem administration there will be blood samples 4h, 8h, 12h, 24 hours, 48h and 72 hours after administration (each of these samples is a maximum of 11 ml of blood (a 1 tablespoon). These samples will allow to obtain information regarding your blood clotting function.
An echography of your heart will be performed after the 1st administration and an echography of your liver will be done before each infusion, 2 and 4 weeks after the 1st administration.

During the administration of HepaStem, a representative of the sponsor may be present to provide additional information and advice to your study doctor, who remains responsible for the administration. This representative is obliged to follow all rules of confidentiality and protection of the privacy stipulated below.

3 Follow up period: during this period, we will ask you to come back to the hospital 2 months, 3 months, 6 months and 12 months after the 1st cell infusion. At these visits, you will have a clinical examination, and a blood sample (maximum of 15 mL, approximately 1 tablespoon of blood). The results of these examinations will be collected.
The blood samples will allow us to analyse parameters that will inform us on the status of your organs (liver, kidney, blood).
During this period, if you have a liver transplantation, if possible, a sample of your explanted liver will be collected.

After the follow up period, we will ask you if you agree to be a part of our registry for additional and regular follow up.

Risks and discomforts

A: Side effects of the study medicinal product :

All medicinal products have known or unforeseeable side effects. Even if previous studies have shown that HepaStem was well tolerated, you may still experience the following side effects: **at short-term:**

- thrombosis: This means formation of a blood clot inside a blood vessel obstructing the flow in this vessel.

- bleeding: in this study, among patients previously treated, two very serious cases of bleeding were observed. Therefore, careful control of your blood clotting parameters will be performed to avoid that.

- respiratory difficulties may occur

- hypersensitivity reaction or reaction to the infusion: This occurs when the body's immune system over reacts to something like medication. Hypersensitivity reactions may include: skin irritation, redness, itching, swelling, fluid discharge, crusting, skin rash, eruptions, coughing or shortness of breath, hoarse voice, headache, clogged or runny nose, sneezing, red (bloodshot) eyes, stomach pain, nausea, vomiting, diarrhea, fatigue, sore throat, dizziness. These reactions may be harmful, uncomfortable or in some cases fatal (in the case of anaphylaxis).

In the medium or long term:

- distribution in different organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of cells from another person which may eventually lead to cell rejection.

Other currently unknown risks and discomforts could appear. It is therefore very important that you report quickly any new health problem to the investigator, regardless of whether or not you think it has to do with the study.

C: Risks associated with the procedures specific to the study

There are also risks/discomforts associated with the specific examinations that will be performed for the study:

- The intravenous administration may cause
    - Pain, as for any injection.
    - Infection. Any break in the skin carries a risk of infection although intravenous insertion is an aseptic procedure.
    - Phlebitis: inflammation of a vein that may be caused by infection.
    - Infiltration: infiltration occurs when a fluid or medication administered in the vein accidentally enters the surrounding tissue rather than the vein.
    - Embolism: A blood clot or other solid mass, or an air bubble, can be delivered into the circulation through an IV and end up blocking a vessel. However, it is nearly impossible to inject air through a peripheral IV. The risk is greater with a central IV.
- The blood sampling necessary for analysis may (rarely) cause pain, bleeding, bruising or infection localised around the puncture site. Similarly, some patients may feel dizzy or even faint during the procedure.

The staff who takes the blood will use best medical care to prevent or to keep these discomforts to a minimum.

Notification of new information

It may be that during the course of a clinical study, important new information on HepaStem being investigated becomes available. You will be informed in a timely manner of any new element that might affect your decision to continue taking part in this study.

If, in the light of the new information, you decide to stop taking part in the study, your investigator will ensure that you continue to receive the best possible treatment.

Contraception, pregnancy and breast-feeding

Female participant**:** Because the effects of HepaStem on an unborn child or infant are not properly known, you will not be allowed to take part in this clinical study if you are pregnant, wish to become pregnant or if you are breast-feeding.

If you choose to take part in this study, you must use one of the authorised methods of contraception (so that you do not become pregnant). Your doctor will discuss the various appropriate options with you.

Benefits

If you agree to take part in this study, HepaStem may or may not prove beneficial in treating your disease or relieving your symptoms, especially helping to resolve the current acute decompensation of liver function.

The information obtained with this study may contribute to a better knowledge of the use of this medicinal product or to the development of a new medicinal product for the treatment of Acute on Chronic Liver Failure in future patients.

Alternative treatment:

No treatment for your condition that has been approved by the authorities is currently available in Europe.

The only treatment currently available is liver transplantation. However, this treatment is far from ideal due to the shortage of organs for transplantation

Withdrawal from the study

Your participation is voluntary and you are entitled to withdraw from the study at any time without giving reason. Nevertheless, it may be useful for the investigator and for the sponsor of the study to know if you are withdrawing because the constraints of the treatment are too great (too many uncomfortable side effects, for example).

It is also possible that the investigator withdraws you from the study because you are pregnant, because he/she thinks it is better for your health or because he/she finds out that you are not following the instructions given to participants.

Finally, the competent national or international authorities, the ethics committee that initially approved the study or the sponsor may decide to stop the study.

If you withdraw your consent to participate in the study, in order to guarantee the validity of the investigation, the encrypted data will be retained until the point you withdrew. No new data may be sent to the sponsor.

If you withdraw your consent to participate in the study, you can contact the researcher and ask to have the samples not yet used to be destroyed. The results obtained from your samples before you withdrew your consent, remain the property of the study sponsor.

Treatment after stopping the study

In all these situations of withdrawing from the study, but also when the scheduled participation period has ended, your investigator will assess your state of health and prescribe the best treatment available.

Samples of biological material collected during the study

The sponsor of the study assures that the samples (e.g. blood, urine, liver tissue) will only be used within the context of the study.

- The procedure for encrypting samples is the same as the one used for your medical data. Therefore, samples sent to the sponsor will only contain your study ID code.

-The ones who manage these samples (the haematology Laboratory of the Cliniques St. Luc in Brussels, Belgium and the translational research Center, KU Leuven, Leuven, Belgium) will use them within the context of clinical research and destroy them at the end of the planned Retention period.

-The taken sample of biological material is considered a ' donation ' and you should be aware that, in principle, you will not receive any financial benefit (royalties) associated with the development of new therapies derived of the use of your donation of biological material, and which may have commercial value.

The surplus of your samples will be destroyed once the analyses described in this document have been carried out (at the latest, one year after the end of the study)

Confidentiality and data protection

Your participation in the study means that you agree that the researcher collects data about you and that the study sponsor uses this data for research purposes and for scientific and medical publications.

You have the right to ask the researcher what information is collected about you and for what they are used in function of the study. These data include your current clinical situation, but also some background data, the results of surveys conducted within the context of your health care in accordance with current standards and of course the results of evaluations required by the Protocol. You have the right to review and correct this data if they are wrong. These rights are guaranteed by the [local country law about data protection to be mentioned here]

The researcher is bound by the professional secrecy with regard to the data collected.

This means that he/she is obliged to never disclose your name in the context of a publication or conference and that he/she will also encrypt your data (your identity will be replaced by an ID code in the study) before sending collected data to the database administrator (Clinical Department of Promethera Bioscience).

The researcher and his/her team will therefore be the only ones who can link the data sent during the study and your medical dossier. For this study, the law requires that this link is retained with your dossier for at least 30 years and for a maximum of 50 years, in accordance with [local country law to be mentioned here]

The personal information that is sent will not contain any combination of elements from which you can be identified.

For the administrator of the study data that is indicated by the sponsor, the data sent will not allow you to identify. The Administrator is responsible for collecting the data of all researchers who participate in this study and to process and protect them in accordance with the requirements of the Belgian Law on the protection of privacy.

In order to verify the quality of the study, it is possible that your medical dossier is examined by persons bound by the professional secrecy and designated by the Ethics Committee, the sponsor of the study or an independent supervisory body. In any case, this examination of your medical dossier may only take place under the responsibility of the researcher and supervised by one of the employees appointed by him/her.

The (coded) study data may be sent to national regulatory authorities, the relevant ethics committees, other doctors and/or organisations that collaborate with the sponsor.

They may also be sent to other branches of the sponsor in Belgium and other countries where the standards for protecting personal data may be different or less stringent. As explained above, the data sent is encrypted. The sponsor undertakes to respect the limitations of the European directive and the Belgian legislation on the protection of privacy.

Your consent to participate in this study implies your consent to the use of your coded medical data for the purpose described in this information form and for its transfer to the above-mentioned persons and Authorities. The sponsor agrees to use the data only in the context of the study in which you participate.

Insurance:

Any participation in a clinical study involves a risk, however small. The sponsor is, even without fault, liable for the damage which the subject and his rightful claimants (in case of death) sustained and which shows either a direct or an indirect connection with the trial. An insurance covering this liability has been established. You are therefore asked to report the investigator of any new health problems before consulting another doctor, taking another medication or receiving any other medical treatment. If for any reason you consult another physician during this clinical trial, you must inform them that you are participating in a clinical trial/present them with your clinical trial participant card. This can be important for diagnosing and handling your complaints.

If the investigator believes a link to the study is possible (the insurance does not cover the natural progression of your illness or the known side effects of your normal treatment), he/she will inform the Sponsor who will initiate the reporting procedure to the insurance company. They will appoint an expert - if they consider it necessary - to determine if there is a link between your new health problem and the study.

In the event of a disagreement with the investigator or expert appointed by the insurance company, as well as whenever you consider it useful, you or - in the event of death - your beneficiaries can initiate proceedings against the insurer directly in [Country] [name of the insurance company, policy number, contact person]. The law ensures that the insurer can be summoned by the judge of the place where the incident giving rise to the damage occurred, or before the judge of your domicile, or before the judge of the head office of the insurer.

If you take part in this clinical study, we ask you:

- To not drink alcohol for at least 5 weeks from the start of your participation.
- To cooperate fully in the smooth running of this study.
- Not to conceal any information relating to your state of health, the medication you are taking or the symptoms you are experiencing.
- Not to take part in other clinical study involving an investigational treatment, be it a medicinal product, a medical device or a procedure, while taking part in this study.
- To carry the "emergency card" with you at all times. This is imperative for your safety in the event of emergency care in an institution that does not know you.

Contact

If you need further information, but also if you have problems or concerns, you can contact the investigator ……..or a member of his/her research team..... on the following telephone number (xx / xxx-xx-yy) ……..

In case of emergency or outside consulting hours, you can contact the emergency department…… on the following telephone number.....indicating that you are taking part in a clinical study. Your records will contain information of use to the on-call doctor in relation to this clinical study.

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HEP101 STUDY

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Patient Informed Consent

Study Patient ID : ……..

Participant

I declare that I have been informed of the nature of the study, its purpose, its duration, any risks and benefits and what is expected of me. I have taken note of the information document I have had sufficient time to think about it and discuss it with a person of my choice, such as my general practitioner (GP) or a member of my family.

I have had the opportunity to ask the investigator any questions that came to mind and have obtained a satisfactory response to my questions.

I understand that my participation in this study is voluntary and that I am free to end my participation in this study without this affecting my relationship with the therapeutic team in charge of my health.

I am aware that for this clinical study, personal data (especially my medical records) be collected, stored and analysed. The use of the information concerning my health is in accordance with legal provisions and requires a voluntarily given informed consent prior to my participation in this study. Without the subsequent agreement I cannot participate in the study.
Personal data is encrypted. I have the right to review/correct my data.

The consent to the collection and processing of my personal data, especially information about my health is irrevocable. I have already been informed that I can stop participating in this trial at any time. In case of such withdrawal of my consent, I agree that data collected until this point can be analyzed pseudonymously (encrypted).

I agree that my data will be kept after the end of the study or discontinuation of the study, in accordance with the required national requirements.

I agree to the study data collected in order to achieve the objectives of this study may be further processed, provided that such treatment is limited to the context of the present study for a better understanding of the disease and its treatment.

I agree to the sponsor retaining samples of biological material collected during the course of the study after the completion of the clinical study for subsequent research purposes, but limited to the context of this study.

I agree my GP or other specialists in charge of my health, being informed of my participation in this clinical study.

I have received a copy of the information to the participant and the informed consent form.

Patient's First Name: ……Name: ……

Signature: …… Date: ……

Legal representative

I declare that I am being asked to take a decision on whether or not to take part in the clinical study for the person I represent in his/her best interests and taking into consideration his/her likely wishes. My consent applies to all the items listed in the consent of the participant.

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I have received a copy of the information to the participant and the informed consent form.

legal representative's First Name: ……Name ……

Signature: …… Date: ……

Witness/Interpreter

I was present during the entire process of informing the patient and I confirm that the information on the objectives and procedures of the study was adequately provided, that the participant (or his/her legal representative) apparently understood the study and that consent to participate in the study was freely given.

the witness/interpreter: … First Name & Name: ……..

Signature: …… Date : ……

Investigator

I, the undersigned,……., investigator, confirm that I have verbally provided the necessary information about the study and have given the participant a copy of the information document.

I confirm that no pressure was applied to persuade the patient to agree to take part in the study and that I am willing to answer any additional questions if required.

I confirm that I operate in accordance with the ethical principles set out in the latest version of the "Helsinki Declaration", the "Good Clinical Practices" and current national regulation related to trials that involve the participation of human subjects.

First Name of the investigator: ……Name……

Signature: …… Date : ……

HEP101 STUDY

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Retrospective Information

Given your clinical condition when you were admitted to intensive care, you were unable to decide for yourself whether or not to take part in the above-mentioned study.

It is then customary to use a legal representative (usually a close relative), who is asked to make a decision on the person's participation in the study in the best interests of this person and taking into consideration his/her likely wishes.

You should be aware that the ethics committee agreed to the application to this study of the emergency procedure on .....

You were therefore included in this study, you agreed to participate, but, not being fully capable of deciding for yourself, the investigator asked your legal representative to confirm your participation by signing the consent.

Now, improving your clinical situation allows you to be properly informed about the objectives and procedures of the study and give your consent to continue or discontinue your participation in the study.

We are now asking you to confirm your wish to continue or discontinue this participation, and we invite you to take note of the document informing you fully on the objectives and procedures of the study, as well as on the possible risks and benefits of the investigational treatment and your rights as a participant in a clinical study.

Please read these few pages of information carefully and ask any questions you want to the investigator or his/her representative. There are 2 parts to this document: the information essential to your decision and your written consent.

In this case, a new informed consent will be re-signed by you and the investigator of the study.

HEP101 STUDY

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Patient Information

This patient information and the informed consent form are for the patients or legal representatives of patients suffering from Acute-on-Chronic Liver Failure (ACLF) who are being invited to participate in a research with HepaStem

Sponsor: **Promethera Biosciences**
Address: **Rue Granbonpré 11**
1435 Mont St Guibert
Belgium

Principal Investigator: _____
Address _____
Telephone number: _____

Introduction

You are being invited to take part in a clinical study to evaluate an investigational medicinal product for the treatment of your disease. An investigational medicinal product is a medicinal product that is still being studied to evaluate its efficacy, safety or mode of action.

For the attention of the patient: It may be that when you were included in the study, you were not fully capable to decide for yourself whether or not to take part in this study. It is then customary to use a legal representative, who is asked to make a decision on the person's participation in the study in the best interests of this person and taking into consideration his/her likely wishes.

For the attention of the legal representative: Because of his/her clinical situation, we consider that the person you represent is able to decide whether or not to participate in the study, although on the occasion of his/her illness it could be questioned that they are fully aware of the implications of the study. For this reason, you are invited to decide whether the person you represent should participate in this clinical trial, taking into consideration his/her likely wishes.

In the remainder of this document, the sentences are worded as if we were directly addressing the person you represent.

The Investigator (or study doctor who is the physician responsible for this research study at the hospital) and the Sponsor (company which initiates and finances this research) expect that this medicinal product offers advantages in the treatment of patients with the same disease as yours. There is, however, no guarantee that you will benefit from taking part in this study.

Before you agree to take part in this study, we invite you to take note of its implications in terms of organisation, possible risks and benefits, to allow you to make a decision with full awareness of the implications. This is known as giving "informed consent".

Please read this information carefully and ask any questions you want to the investigator or his/her representative. There are 2 parts to this document: the information essential to your decision, and your written consent form.

Before you make your decision, you should be aware that:

- Before its start-up, this clinical study has been the subject of an evaluation by an ethics committee ([national EC to be mentioned]) after consulting the ethics committees of other participating centers. In any case, you should not consider the favorable opinion of that committee as an incitement to participate in this study.
- Your participation is voluntary and must remain free from any coercion. It requires the signature of a document expressing your consent. Even after having signed this document, you can stop taking part by informing the investigator. Your decision not to take part or to stop taking part in the study will have no impact on the quality of your care or on your relationship with the investigator.
- The data collected in the study are confidential and your anonymity is always guaranteed including during publication of the results.
- The sponsor has contracted an insurance policy in case you should suffer any damage in connection with your participation in this clinical study.

  Insurance company: [to be completed for each country]
  Policy number: [to be completed for each country]
- Your participation in this study will be totally free. No cost will be charged for the exams performed within this study. However, you will not be offered any remuneration for participating in this study.
- Expenses related to your travel to the hospital (such as your vehicule's expenses, taxis, train tickets, etc.) will be reimbursed [please precise the way of reimbursement (vouchers/payment by hospital]
- The sponsor will reimburse the hospital/investigator for all the expenses associated with the visits/consultations, the exams and the treatments specifically related to the study.
- You may contact the investigator or a member of his/her team any time should you need any additional information.

Objectives and description of the study protocol

We are inviting you to take part in a clinical study involving human hepatic cells (called progenitor cells) derived from donated livers. These cells are expected to have a combined systemic (in your whole body) and local effect in the liver. They are expected to play an immunomodulatory role, meaning that they help regulating the exacerbated inflammatory response observed in your disease and especially help resolving the current acute event. The cells being tested are called Heterologous (from another person) Human Adult Liver-derived Progenitor Cells (HHALPC) or HepaStem.
The cells are stem cells isolated from adult livers and are not embryonic stem cells. They are preserved in a specific way, and are approved by the health authorities to be used in patients in a clinical trial setting.

Approximately twenty-one (21) patients will be enrolled in this study in Europe, including approximately [number of patient expected in local country] in [local country]. The investigator will propose to participate to patients, who, like you, have a diagnosed cirrhosis and Acute on Chronic Liver Failure.

The main objective of this study is to assess the safety and efficacy of multiple (2) dosages of HepaStem administered in a single or repeated doses, with an interval of minimum 1 week.

This study is an open study of various dose regimens. This means you will either receive

- a single low-dose administration of 0.25 million cells per kilogram, with a maximum of 25 million of cells per infusion (which implies the administration of a maximum 5 ml syringe); Or else,
- a single dose of 0.50 million of cells per kilogram, with a maximum of 50 million of cells per infusion (which implies the administration of a maximum 10 ml syringe); Or else

a single dose of 1.0 million of cells per kilogram, with a maximum of 100 million of cells per infusion (which implies the administration of a maximum 20 ml syringe); Or else,an administration followed by a second administration one week later at least. The dosage of the second administration will be equal to the first administered dosage. A dose of 0.50 million of cells per kilogram with a maximum of 50 million per infusion (which implies the administration of a maximum 10 ml syringe) or a dose of 1.0 million of cells per kilogram with a maximum of 100 million per infusion (which implies the administration of a maximum 20 ml syringe)will be administered.

In all cases, you and your research doctor will know the dosage you are receiving.

Course of the study

After the treatment with cells and further 3 to 4 weeks of surveillance, you will remain in the study until 1 year is over (follow-up period). Within the first month you will have 8 study assessments, i.e. days with study-specific procedures and examinations. During the follow-up period, you will have 4 study assessments.

The examinations or procedures that are required in connection with the study are described below. Some of the examinations and procedures are part of the standard care provided in your hospital, while others are done specifically for the study.

Your participation in the study will include 3 Period:

1 Screening Period: during which, your investigator will verify that you are eligible to participate in this study and will collect your medical history and the data of some of the examinations that have been performed after you agreed to participate in the study.

A maximum of 32 mL (approximately 3 tablespoons of blood) will be taken. Blood samples are already done as part of the care of your current clinical situation; therefore, no additional puncture on your arm will be done to collect these blood tubes.

Some analysis of your urine will be also done.

The blood and urine samples will allow us to analyse parameters that will inform us on the status of your organs (liver, kidney, blood), on your virology status (including Hepatitis and HIV), and on your immunity.

If it has not been already done, you will have an echography of your liver and your heart, a chest X ray and an electrocardiogram. These examinations are not invasive and won't be painful.

You will receive an "emergency card" with all information regarding the study. Please keep this card with you any time. If you're hospitalized, please show this card to your doctor and inform him/her that you are participating in this study

After the screening period, if all criteria to enter the study are met you will be able to start the active study period and receive the HepaStem.

2 Active Period: During this period, you will be given 1 or 2 doses of HepaStem. Depending on the group you belong to, you will be given 0.25 million cells per kilogram (with a maximum of 25 million cells per infusion in case your weight is above 100 kilogram), 0.5 million cells per kilogram (with a maximum of 50 million cells per infusion in case your weight is above 100 kilogram) or 1.0 million cells per kilogram (with a maximum of 100 million cells per infusion in case your weight is above 100 kilogram). This corresponds to a volume of 5, 10 or 20 mL liquid. HepaStem will be administered in an intravenous way, which means that a needle will be put in a small or large vein to administer the product. Medication will be given before HepaStem infusion to prevent adverse reactions of the body to the product.

During each administration and in any case 4, 8 and 12 days after and 2, 3 and 4 weeks after your 1st administration, you will have clinical examinations and blood samples will be taken (maximum of 25 mL, approximately 2 tablespoons of blood). The blood samples will allow to gain information on the status of your organs (liver, kidney, blood).
During each HepaStem administration there will be blood samples 4h, 8h, 12h, 24 hours, 48h and 72 hours after administration (each of these samples is a maximum of 11 ml of blood (a 1 tablespoon). These samples will allow to obtain information regarding your blood clotting function.
An echography of your heart will be performed after the 1st administration and an echography of your liver will be done before each infusion, 2 and 4 weeks after the 1st administration.

During the administration of HepaStem, a representative of the sponsor may be present to provide additional information and advice to your study doctor, who remains responsible for the administration. This representative is obliged to follow all rules of confidentiality and protection of the privacy stipulated below.

3 Follow up period: during this period, we will ask you to come back to the hospital 2 months, 3 months, 6 months and 12 months after the 1st cell infusion. At these visits, you will have a clinical examination, and a blood sample (maximum of 15 mL, approximately 1 tablespoon of blood). The results of these examinations will be collected.
The blood samples will allow us to analyse parameters that will inform us on the status of your organs (liver, kidney, blood).
During this period, if you have a liver transplantation, if possible, a sample of your explanted liver will be collected.

After the follow up period, we will ask you if you agree to be a part of our registry for additional and regular follow up.

Risks and discomforts

A: Side effects of the study medicinal product :

All medicinal products have known or unforeseeable side effects. Even if previous studies have shown that HepaStem was well tolerated, you may still experience the following side effects: **at short-term:**

- thrombosis: This means formation of a blood clot inside a blood vessel obstructing the flow in this vessel.

- bleeding: in this study, among patients previously treated, two very serious cases of bleeding were observed. Therefore, careful control of your blood clotting parameters will be performed to avoid that.

- respiratory difficulties may occur

- hypersensitivity reaction or reaction to the infusion: This occurs when the body's immune system over reacts to something like medication. Hypersensitivity reactions may include: skin irritation, redness, itching, swelling, fluid discharge, crusting, skin rash, eruptions, coughing or shortness of breath, hoarse voice, headache, clogged or runny nose, sneezing, red (bloodshot) eyes, stomach pain, nausea, vomiting, diarrhea, fatigue, sore throat, dizziness. These reactions may be harmful, uncomfortable or in some cases fatal (in the case of anaphylaxis).

In the medium or long term:

- distribution in different organs where cells may promote tumoral development although such events have been rarely reported with cell therapy, immune response as HepaStem is made of cells from another person which may eventually lead to cell rejection.

Other currently unknown risks and discomforts could appear. It is therefore very important that you report quickly any new health problem to the investigator, regardless of whether or not you think it has to do with the study.

C: Risks associated with the procedures specific to the study

There are also risks/discomforts associated with the specific examinations that will be performed for the study:

- The intravenous administration may cause
    - Pain, as for any injection.
    - Infection. Any break in the skin carries a risk of infection although intravenous insertion is an aseptic procedure.
    - Phlebitis: inflammation of a vein that may be caused by infection.
    - Infiltration: infiltration occurs when a fluid or medication administered in the vein accidentally enters the surrounding tissue rather than the vein.
    - Embolism: A blood clot or other solid mass, or an air bubble, can be delivered into the circulation through an IV and end up blocking a vessel. However, it is nearly impossible to inject air through a peripheral IV. The risk is greater with a central IV.
- The blood sampling necessary for analysis may (rarely) cause pain, bleeding, bruising or infection localised around the puncture site. Similarly, some patients may feel dizzy or even faint during the procedure.

The staff who takes the blood will use best medical care to prevent or to keep these discomforts to a minimum.

Notification of new information

It may be that during the course of a clinical study, important new information on HepaStem being investigated becomes available. You will be informed in a timely manner of any new element that might affect your decision to continue taking part in this study.

If, in the light of the new information, you decide to stop taking part in the study, your investigator will ensure that you continue to receive the best possible treatment.

Contraception, pregnancy and breast-feeding

Female participant**:** Because the effects of HepaStem on an unborn child or infant are not properly known, you will not be allowed to take part in this clinical study if you are pregnant, wish to become pregnant or if you are breast-feeding.

If you choose to take part in this study, you must use one of the authorised methods of contraception (so that you do not become pregnant). Your doctor will discuss the various appropriate options with you.

Benefits

If you agree to take part in this study, HepaStem may or may not prove beneficial in treating your disease or relieving your symptoms, especially helping to resolve the current acute decompensation of liver function.

The information obtained with this study may contribute to a better knowledge of the use of this medicinal product or to the development of a new medicinal product for the treatment of Acute on Chronic Liver Failure in future patients.

Alternative treatment:

No treatment for your condition that has been approved by the authorities is currently available in Europe.

The only treatment currently available is liver transplantation. However, this treatment is far from ideal due to the shortage of organs for transplantation

Withdrawal from the study

Your participation is voluntary and you are entitled to withdraw from the study at any time without giving reason. Nevertheless, it may be useful for the investigator and for the sponsor of the study to know if you are withdrawing because the constraints of the treatment are too great (too many uncomfortable side effects, for example).

It is also possible that the investigator withdraws you from the study because you are pregnant, because he/she thinks it is better for your health or because he/she finds out that you are not following the instructions given to participants.

Finally, the competent national or international authorities, the ethics committee that initially approved the study or the sponsor may decide to stop the study.

If you withdraw your consent to participate in the study, in order to guarantee the validity of the investigation, the encrypted data will be retained until the point you withdrew. No new data may be sent to the sponsor.

If you withdraw your consent to participate in the study, you can contact the researcher and ask to have the samples not yet used to be destroyed. The results obtained from your samples before you withdrew your consent, remain the property of the study sponsor.

Treatment after stopping the study

In all these situations of withdrawing from the study, but also when the scheduled participation period has ended, your investigator will assess your state of health and prescribe the best treatment available.

Samples of biological material collected during the study

The sponsor of the study assures that the samples (e.g. blood, urine, liver tissue) will only be used within the context of the study.

- The procedure for encrypting samples is the same as the one used for your medical data. Therefore, samples sent to the sponsor will only contain your study ID code.

-The ones who manage these samples (the haematology Laboratory of the Cliniques St. Luc in Brussels, Belgium and the translational research Center, KU Leuven, Leuven, Belgium) will use them within the context of clinical research and destroy them at the end of the planned Retention period.

-The taken sample of biological material is considered a ' donation ' and you should be aware that, in principle, you will not receive any financial benefit (royalties) associated with the development of new therapies derived of the use of your donation of biological material, and which may have commercial value.

The surplus of your samples will be destroyed once the analyses described in this document have been carried out (at the latest, one year after the end of the study)

Confidentiality and data protection

Your participation in the study means that you agree that the researcher collects data about you and that the study sponsor uses this data for research purposes and for scientific and medical publications.

You have the right to ask the researcher what information is collected about you and for what they are used in function of the study. These data include your current clinical situation, but also some background data, the results of surveys conducted within the context of your health care in accordance with current standards and of course the results of evaluations required by the Protocol. You have the right to review and correct this data if they are wrong. These rights are guaranteed by the [local country law about data protection to be mentioned here]

The researcher is bound by the professional secrecy with regard to the data collected.

This means that he/she is obliged to never disclose your name in the context of a publication or conference and that he/she will also encrypt your data (your identity will be replaced by an ID code in the study) before sending collected data to the database administrator (Clinical Department of Promethera Bioscience).

The researcher and his/her team will therefore be the only ones who can link the data sent during the study and your medical dossier. For this study, the law requires that this link is retained with your dossier for at least 30 years and for a maximum of 50 years, in accordance with [local country law to be mentioned here]

The personal information that is sent will not contain any combination of elements from which you can be identified.

For the administrator of the study data that is indicated by the sponsor, the data sent will not allow you to identify. The Administrator is responsible for collecting the data of all researchers who participate in this study and to process and protect them in accordance with the requirements of the Belgian Law on the protection of privacy.

In order to verify the quality of the study, it is possible that your medical dossier is examined by persons bound by the professional secrecy and designated by the Ethics Committee, the sponsor of the study or an independent supervisory body. In any case, this examination of your medical dossier may only take place under the responsibility of the researcher and supervised by one of the employees appointed by him/her.

The (coded) study data may be sent to national regulatory authorities, the relevant ethics committees, other doctors and/or organisations that collaborate with the sponsor.

They may also be sent to other branches of the sponsor in Belgium and other countries where the standards for protecting personal data may be different or less stringent. As explained above, the data sent is encrypted. The sponsor undertakes to respect the limitations of the European directive and the Belgian legislation on the protection of privacy.

Your consent to participate in this study implies your consent to the use of your coded medical data for the purpose described in this information form and for its transfer to the above-mentioned persons and Authorities. The sponsor agrees to use the data only in the context of the study in which you participate.

Insurance:

Any participation in a clinical study involves a risk, however small. The sponsor is, even without fault, liable for the damage which the subject and his rightful claimants (in case of death) sustained and which shows either a direct or an indirect connection with the trial. An insurance covering this liability has been established. You are therefore asked to report the investigator of any new health problems before consulting another doctor, taking another medication or receiving any other medical treatment. If for any reason you consult another physician during this clinical trial, you must inform them that you are participating in a clinical trial/present them with your clinical trial participant card. This can be important for diagnosing and handling your complaints.

If the investigator believes a link to the study is possible (the insurance does not cover the natural progression of your illness or the known side effects of your normal treatment), he/she will inform the Sponsor who will initiate the reporting procedure to the insurance company. They will appoint an expert - if they consider it necessary - to determine if there is a link between your new health problem and the study.

In the event of a disagreement with the investigator or expert appointed by the insurance company, as well as whenever you consider it useful, you or - in the event of death - your beneficiaries can initiate proceedings against the insurer directly in [Country] [name of the insurance company, policy number, contact person]. The law ensures that the insurer can be summoned by the judge of the place where the incident giving rise to the damage occurred, or before the judge of your domicile, or before the judge of the head office of the insurer.

If you take part in this clinical study, we ask you:

- To not drink alcohol for at least 5 weeks from the start of your participation.
- To cooperate fully in the smooth running of this study.
- Not to conceal any information relating to your state of health, the medication you are taking or the symptoms you are experiencing.
- Not to take part in other clinical study involving an investigational treatment, be it a medicinal product, a medical device or a procedure, while taking part in this study.
- To carry the "emergency card" with you at all times. This is imperative for your safety in the event of emergency care in an institution that does not know you.

Contact

If you need further information, but also if you have problems or concerns, you can contact the investigator ……..or a member of his/her research team..... on the following telephone number (xx / xxx-xx-yy) ……..

In case of emergency or outside consulting hours, you can contact the emergency department…… on the following telephone number.....indicating that you are taking part in a clinical study. Your records will contain information of use to the on-call doctor in relation to this clinical study.

,

HEP101 STUDY

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Patient Informed Consent

Study Patient ID : ……..

Participant

I declare that I have been informed of the nature of the study, its purpose, its duration, any risks and benefits and what is expected of me. I have taken note of the information document I have had sufficient time to think about it and discuss it with a person of my choice, such as my general practitioner (GP) or a member of my family.

I have had the opportunity to ask the investigator any questions that came to mind and have obtained a satisfactory response to my questions.

I understand that my participation in this study is voluntary and that I am free to end my participation in this study without this affecting my relationship with the therapeutic team in charge of my health.

I am aware that for this clinical study, personal data (especially my medical records) be collected, stored and analysed. The use of the information concerning my health is in accordance with legal provisions and requires a voluntarily given informed consent prior to my participation in this study. Without the subsequent agreement I cannot participate in the study.
Personal data is encrypted. I have the right to review/correct my data.

The consent to the collection and processing of my personal data, especially information about my health is irrevocable. I have already been informed that I can stop participating in this trial at any time. In case of such withdrawal of my consent, I agree that data collected until this point can be analyzed pseudonymously (encrypted).

I agree that my data will be kept after the end of the study or discontinuation of the study, in accordance with the required national requirements.

I agree to the study data collected in order to achieve the objectives of this study may be further processed, provided that such treatment is limited to the context of the present study for a better understanding of the disease and its treatment.

I agree to the sponsor retaining samples of biological material collected during the course of the study after the completion of the clinical study for subsequent research purposes, but limited to the context of this study.

I agree my GP or other specialists in charge of my health, being informed of my participation in this clinical study.

I have received a copy of the information to the participant and the informed consent form.

Patient's First Name: ……Name: ……

Signature: …… Date: ……

Legal representative

I declare that I am being asked to take a decision on whether or not to take part in the clinical study for the person I represent in his/her best interests and taking into consideration his/her likely wishes. My consent applies to all the items listed in the consent of the participant.

.

I have received a copy of the information to the participant and the informed consent form.

legal representative's First Name: ……Name ……

Signature: …… Date: ……

Witness/Interpreter

I was present during the entire process of informing the patient and I confirm that the information on the objectives and procedures of the study was adequately provided, that the participant (or his/her legal representative) apparently understood the study and that consent to participate in the study was freely given.

the witness/interpreter: … First Name & Name: ……..

Signature: …… Date : ……

Investigator

I, the undersigned,……., investigator, confirm that I have verbally provided the necessary information about the study and have given the participant a copy of the information document.

I confirm that no pressure was applied to persuade the patient to agree to take part in the study and that I am willing to answer any additional questions if required.

I confirm that I operate in accordance with the ethical principles set out in the latest version of the "Helsinki Declaration", the "Good Clinical Practices" and current national regulation related to trials that involve the participation of human subjects.

First Name of the investigator: ……Name……

Signature: …… Date : ……

HEP101 STUDY

MULTICENTER PHASE II SAFETY AND PRELIMINARY EFFICACY STUDY OF 2 DOSE REGIMENS OF HEPASTEM IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

Retrospective Information

Given your clinical condition when you were admitted to intensive care, you were unable to decide for yourself whether or not to take part in the above-mentioned study.

It is then customary to use a legal representative (usually a close relative), who is asked to make a decision on the person's participation in the study in the best interests of this person and taking into consideration his/her likely wishes.

You should be aware that the ethics committee agreed to the application to this study of the emergency procedure on ……..

You were therefore included in this study, you agreed to participate, but, not being fully capable of deciding for yourself, the investigator asked your legal representative to confirm your participation by signing the consent.

Now, improving your clinical situation allows you to be properly informed about the objectives and procedures of the study and give your consent to continue or discontinue your participation in the study.

We are now asking you to confirm your wish to continue or discontinue this participation, and we invite you to take note of the document informing you fully on the objectives and procedures of the study, as well as on the possible risks and benefits of the investigational treatment and your rights as a participant in a clinical study.

Please read these few pages of information carefully and ask any questions you want to the investigator or his/her representative. There are 2 parts to this document: the information essential to your decision and your written consent.

In this case, a new informed consent will be re-signed by you and the investigator of the study.

federal agency for ,incl hci1ltl1 product
Research and Development

22 JUIL. 2016

Federal agency for medicines and health products
Eurostation II - Place Victor Horta 40/40
1060 Bruxelles
www .afmps.b e

Iris Zwaenepoel
Tel. : + 32 (0)2 528 42 08
Fax : + 32 (0)2 528 40 01
e-mail : iris.zwaenepoel@fagg.be

Promethera Biosciences
Carole de Meester
Rue Granbonpre, 11
1435 Mont-Saint-Guibert

Your letter from	Your reference	Our reference	Annex	Oat
		FAGG/R&D/ISZ/kvk \C\'5 G,S. -		8 JU\I. 1.016

Onderwerp Goedkeuring van een klinische proef op 30 juni 2016 .
Titre de l'objet Approbation d'un essai clinique le 30 juin 2016.
Subject Authorisation of a clinical trial dated 30 June 2016 .

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute on Chronic Liver Failure

EudraCT : 2016-001177-32

Chere Madame, Cher Monsieur,

Conformement a !'article 12 de la Loi du 7 mai 2004 relative aux experimentations sur la personne humaine, j'ai decide d'autoriser l'essai clinique ci-dessus mentionne.

Salutations sinceres,

Pour la Ministre des Affaires sociales et de la Sante publique

Geachte Mevrouw, Geachte Heer,

In overeenstemming met artikel 12 van de wet van 7 mei 2004 inzake experimenten op de menselijke persoon, heb ik besloten de hierboven vermelde klinische proef goed te ki:>1iri:>n.

Met de meeste hoogachting,

Voor de Minister van Sociale zaken en volksgezondheid

Dr. Greet Musch

Unofficial translation :

In accordance with article 12 of the Law of 7 May 2004 concern ing experiments on the human person, I have decided to authorise the above mentioned clinical trial .

Comité de Protection des Personnes
Ile-de-France VII
BICETRE
Hôpital de Bicêtre – 78 rue du général Leclerc – 94275 Le Kremlin Bicêtre Cedex
Chair : Françoise BOISSY — Secretary : Brigitte PILATE

Project research Number: 16-014 Kremlin Bicêtre on July 26th, 2016

The Committee had before it on 22 April 2016:

By Mrs BARTHEL on behalf of the Sponsor PROMETHERA BIOSCIENCES - rue Granbonpré, 1 1 - 1435 MONT SAINT GUILBERT - BELGIUM concerning the research project entitled:

"Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure."

Sponsor Reference: HEP101 – EudraCT Number: 2016-001177-32
For which the coordinating investigators is Professor Samuel, Centre Hepato-Biliaire, Paul Brousse Hospital, 12 Avenue Paul Vaillant Couturier, 94804 Villejuif, Cedex.

The Ethics Committee has reviewed the information relating to this project (protocol version 1.1 dated May 25th, 2016 and synopsis version 1.1 dated May 25th, 2016) and the answers you provided to the questions of the first review during the meeting of May 11th, 2016 and June 08th, 2016 with the Investigator Brochure, version 1.0 dated March 25th, 2016, the French Clinical sites list version 1.0 dated April 12th, 2016, the Case Report Form version draft 0.1 dated April 14th, 2016, the patient card version 1.0 dated May 25th, 2016 and Informed Consent Form version 1.1 dated May 25th, 2016.

Members present during the deliberation of your protocol:
First college
- Biomedical research: Mr. G. de FILIPPO, paediatrician (T), Ms F. FAYARD, epidemiologist (T), Mr. de BEAUREPAIRE (T) and Ms. F. HIRSCH (T).
- Treating Physician: Mr. G. COINDARD
- Pharmacist: Mrs D. BLONDELON (S)

Second College:
- Ethics: Mr. P. CASAURANG (T)
- Caseworker: Mrs M. ORBACH-ROULIERE (T)
- Lawyer: Mrs F. BOISSY (T) and Mrs V. A. LAFOY (T)
- Approved associations: Mr COTTET (T) and Mrs A. LABBE (T)

The Committee:
- Considering the interest of the research project
- Considering the respect of an adapted methodology to the question posed
- Considering the respect of a free and informed consent formulated in the light of an adapted information note
- Considering the benefit-risk ratio for the patient

adopted the following deliberation:

Favourable opinion without restriction

Michel BOTTLAENDER
President of the meeting dated June 08th, 2016

[logo] **UZ LEUVEN**

Leuven, August 9, 2016 [logo]

Medical Ethics Committee
UZ KU Leuven/Research
U.Z. Gasthuisberg
Herestraat 49
B 3000 Leuven (Belgium)

Prof. Dr. Frederik Nevens
INTERNAL MEDICINE,
GASTROINTESTINAL AND LIVER
DISEASES

Our ref.:	EudraCT no.:	Belg. Reg. no.:
S59212	2016-001177-32	

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure.

FINAL FAVORABLE OPINION

Dear Colleague,

The Medical Ethics Committee of UZ KU Leuven/Research studied and discussed the aforementioned protocol at its meeting on June 22, 2016.

After examining the additional information and/or amended documents relating to this file, the Committee believes that the proposed study, as outlined in the protocol, is scientifically relevant and ethically responsible. After consulting the ethics committee of the other participating centers, they also issued a favorable opinion for this study.

When assessing this file, account was taken of the documents and information relating to this study, submitted on May 27, 2016 and August 25, 2016.

This favorable opinion relates to:

Protocol:
Version 1.0 dated March 25, 2016
Information and informed consent form:
ICF version 1.1 160628 NI + F
Other patient documents:
Patient study card: version received 05/27/2016 NI + F
Investigator's brochure/scientific information leaflet:
IB HepaStem (Heterologous Human Adult Liver-derived Progenitor Cells, HHALPC)
Acute-on-Chronic Liver Failure: ed 1.0 dated March 25, 2016
Proof of "no-fault" insurance:
Compiled on April 19, 2016

The Committee confirms that it operates in compliance with the ICH-GCP principles (International Conference on Harmonization Guidelines on Good Clinical Practice), with the most recent version

of the Declaration of Helsinki and with the applicable laws and regulations.

The Committee confirms that in the case of a conflict of interests, the involved members will not participate in the decision-making process for the study.

A membership list is enclosed.

Main points: (if applicable)

The Client is responsible for ensuring that the documents in other languages conform with the Dutch documents.

*If a **Clinical Trial Agreement** is in place, the study can only be carried out in our center once this Clinical Trial Agreement has been approved and signed by the delegated Director of UZ Leuven (and/or by the authorized representative(s) of KU Leuven R&D, if necessary).*

Studies involving medication and some studies involving "medical devices" must be reported to the FAGG by the client.
Studies involving medicines may only be commenced on the proviso that the Minister (FAGG) has not filed any objections within the legal deadlines as stipulated in Art. 13 of the Belgian Law of 05/07/2004 regarding experiments on human subjects.
Legal deadlines (see KB dated 03/17/2009) also apply for certain studies involving medical devices. For more information in this regard, please refer to the website of the FAGG – www.fagg-afmps.be.

Research on embryos in vitro falls under the Law of May 11, 2003. For research of this type, in addition to a favorable opinion of the Ethics Committee, approval by the Federale Commissie voor medisch en wetenschappelijk onderzoek op embryo's in vitro [Federal Committee for Medical and Scientific Research on Embryos in vitro] is required before this research project can be carried out.

Please also take into account the hospital regulations regarding tissue management and the stipulations of the Law of December 19, 2008.

This favorable opinion of the Committee does not mean that it is responsible for the planned study. You will continue to remain responsible for this. What is more, you must ensure that your opinion as an investigator who is involved is shown in publications, reports for the government, etc., resulting from this study. You must report adverse incidents and adverse effects as stipulated by the Belgische Wet aangaande Experimenten op de menselijke person [Belgian Law concerning Experiments on Human Subjects] dated May 7, 2004 (Art. 27 and 28) and the circular 586 from the FAGG.

Please inform us if a study is not carried out or if it has been concluded or prematurely ended (stating the reasons for this).

*If the study is not concluded within one year, the ICH-GCP requires that an **annual progress report** be provided to the Committee.*

*Please notify us of the (premature or planned) end of a study within the deadlines stipulated by law and submit a **Clinical Study Report** to the Committee.*

Yours faithfully,

[*signed*]
Prof. Dr. Minne Casteels
Chair
Medical Ethics Committee, UZ KU Leuven/Research

[stamp: Prof. Dr. Minne Casteels
Chair of the Medical Ethics Committee
UZ KU Leuven/Research]

CC:
FAGG (Federaal Agentschap voor Geneesmiddelen en Gezondheidsproducten) [Federal Agency for Medicines and Health Products]

CTC (Clinical Trial Center UZ Leuven)

External committee(s):

1. The Committee has taken into consideration the opinion provided by the following local committee(s). The Committee also assumes that these centers will accept the study, unless stated otherwise:

Local Committee	*Investigators*
CHU Hôpital Erasme	Prof. Dr. Thierry Gustot
UCL St-Luc	Prof. Dr. Pierre-François Laterre

2. The following committee(s) has (have) issued an unfavorable opinion:

Local Committee	*Investigators*

3. We have not received an opinion from the following committee(s):

Local Committee	*Investigators*

Membership list/composition of the Committee dated June 22, 2016 (date of the last discussion of the file):

Chair	Prof. Dr. em. Ivo De Wever	Surgical Oncology
Vice-Chair	Prof. Dr. em. Guido Verhoeven	Experimental Medicine
Secretariat	Dr. Sabine Graux	Physician
Secretariat	Dr. Sonja Haesendonck	Physician
	Ms. Christine Mathieu	Medical Legislation
	Ms. Els Raets	Nurse
	Ms. Godelieve Goossens	Nurse
	Ms. Hélène De Somer	Nurse
	Dr. José Thomas	Medical Oncology
	Dr. Lut De Groote	General Practitioner
	Prof. Ben Van Calster	Statistics
	Prof. J. R. Thomas	Clinical Pharmacology
	Prof. Dr. Dominique Bullens	Pediatrics
	Prof. Dr. Gregor Verhoef	Hematology
	Prof. Dr. Jan Van Hemelrijck	Anesthesiology
	Prof. Dr. Jan de Hoon	Clinical Pharmacology
	Prof. Dr. Xavier Bossuyt	Immunology
	Prof. Dr. em. Raymond Verhaeghe	Cardiology
	Prof. Dr. em. Willem Daenen	Cardiac Surgery

CLINICAL TRIAL AUTHORIZATION FOR A MEDICINAL PRODUCT FOR HUMAN USE

Page count: 1
(cover sheet included)

Faxing

Date: October 28th, 2016

Clinical Trial details					
Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure.				
Sponsor	Promethera Biosciences			Ref. EC	Not available
Sponsor Reference :	HEP101	EudraCT Number	2016-001177-32	Ref. ANSM	160447A-62

Sender	Recipient (applicant)
ANSM / Drugs Directorate in Oncology, Hematology, Transplantation, Nephrology, Cell Therapy Products, Tissues and Labile Blood Products. Hemovigilance, Labile Blood Products, Cell Therapy and Radiopharmaceuticals Unit	Carole de Meester Promethera Biosciences 32 10 394342
Dossier followed by : Kamel SEDKAOUI Tél : 33 (0) 1 55 87 34 32 / Fax : 33 (0) 1 55 87 36 42 Email : Kamel.sedkaoui ansm.sante.fr	Fax oo 32 10 394301

Request received on	April 22nd, 2016	Additional information received on	April 28th, 2016

Central Ethics Committee recipient in copy	Ile-de-France VII (Le Kremlin-Bicêtre)	Fax 01 45 21 21 45	

Having regard to the Public Health Code and in particular Articles L. 1 123-8, L. 1 123-12 thereof and having regard to the clinical trial authorisation application file sent to the National Agency for the Safety of Medicines and Health Products (ANSM);

Having regard to the additions made by the sponsor on October 26th, 2016 and the protocol of the trial in question, as amended (version 1.3 dated 26/10/2016), at per request of ANSM

The authorisation mentioned in Article L. 1123-8 of the Public Health Code is granted for the clinical trial mentioned above. This authorisation shall be valid for the entire duration of the trial from the date of this approval.

However, in accordance with Article R. 1123-33 of the Public Health Code, this authorisation lapses if the research has not begun within one year.

La Chef produits
Hémovigilance, produits sanguins labiles
thérapie cellulaire et produits radiopharmaceutiques
Isabelle SAINTE-MARIE

I ask you to forward any request for further information concerning this dossier by e-mail to the box: ams*essaiscliniques@ansm.sante.fr. Please note that you can use the Eudralink secure e-mail system for this purpose. When sending these files, I would ask you to ensure that you include the following information in the subject line of the message:
- for the Substantial Amendment sent to the ANSM for information: MS'/ Ref ANSM of the file
- for Substantial Amendment submitted for authorisation or for mixed dossiers (including amendments submitted for authorisation and others for information): MSII Ref ANSM of the dossier

REPUBLIQUE FRANÇAISE

ansm
Agence nationale de sécurité du méziicament et des produits de santé

Attestation for the importation of medicinal products necessary for the performance of biomedical research —AAI

Faxing

Page count : 1
(Cover sheet included)

Sender	National Agency for the Safety of Medicines and Health Products (ANSM) 143-147 boulevard Anatole France — 93285 Saint Denis Cedex		
Recipient (Applicant) Name, address, phone numbe	Promethera Biosciences Watson et Crick Hill Rue Granbonpré, 1 1 B-1435 Mont-Saint-Guibert Belgique 32 10 39 43 11	Fax number	+32 10 39 43 01

The medicinal products listed below, which are necessary for the performance of the biomedical research referred to below, may be imported based on this certificate to be presented at the time of control by customs officials. This certificate is issued in accordance with Articles L. 5124-13, R. 5121-108 and R. 5121-114 of the Public Health Code. It is only valid for medicinal products used only within the framework of this research, authorised by the ANSM (formerly Afssaps) by virtue of article L. 1 123-8 of the aforementioned code, and for the entire duration of this research.

REFERENCES FROM AUTHORIZED BIOMEDICAL RESEARCH			
Title	Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure.		
Sponsor	Promethera Biosciences	EudraCT Number	2016-001177-32

LIST OF IMPORTED DRUGS [1] [2]

Drug Name [3]	Active substance [3]	Code of the drug name [3]	Dosage(s) [4] and pharmaceutical form of the medicinal product
HepaStem	Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC)	HepaStem	Dosage: Patients will be infused with either 1 billion (cohort 1) or 2 billion (cohort 2) HHALPC in total, intravenously over four days of infusion: - Cohort 1: 250 million cells per day. - Cohort 2: 500 million cells per day Pharmaceutical form: suspension for infusion

[1] Experimental drugs and non-experimental drugs used in the clinical trial

[2] Edit additional lines if necessary

[3] Where appropriate

[4] Record on the same line all the dosages of the same pharmaceutical form of the same drug.

Partie réservée à l'ANSNl (date/signature)

This certificate cancels and replaces the one dated: not applicable

28 - 10 _ 2016

La Chef produits
Hémovigilance, produits sanguins labiles
thérapie cellulaire et produits radiopharmaceutiques
Isabelle SAINTE-MARIE

If you do not receive all the pages of this fax, please contact the contact mentioned on the authorisation decision for the biomedical research concerned.

Confidentialité	Confidentiality
Cette transmission est à l'attention exclusive du(des) destinataires ci-dessus mentionné(s) et peut contenir des informations privilégiées et/ou confidentielles. Si vous n'êtes pas le destinataire voulu ou une personne mandatée pour lui remettre cette transmission, vous avez, reçu ce document par erreur et toute utilisation, révélation, copie ou communication de son contenu est interdite. Si vous avez reçu cette transmission par erreur, veuillez nous en informer par téléphone immédiatement et nous retourner le message original par courrier. Merci.	This transmission is intended to the addressee(s) listed above only and may contain preferential or/and confidential information. If you are not the intended recipient, you are hereby notified that you have received the document by mistake and any use disclosure, copying or communication of the content of this transmission is prohibited. If you have received this transmission by mistake, please call us immediately and return the original message by mail. Thank you.

Vall d'Hebron
Hospital

OPINION OF THE ETHICS COMMITTEE FOR CLINICAL RESEARCH WITH MEDICINES

Ms. Mireia Navarro, Secretary of the ETHICS COMMITTEE FOR CLINICAL RESEARCH WITH MEDICINES of the Vall d'Hebron University Hospital,

CERTIFIES

That this Committee has evaluated the following proposal for a clinical trial
Code : HEP101 EudraCT Number : 2016-001177-32
Title: Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure.

SPONSOR: Promethera Biosciences
PROTOCOL: English Version, 4.0 dated February 15th, 2018. Protocol synopsis in Spanish, version 4.0 dated February 15th, 2018.
ICF GENERAL: Version 3.1_07may2018_ESP_ESP
Other ICF: _____

That this Committee has evaluated Part I of the application for authorisation of the trial, has assessed the sponsor's responses to the requested clarifications (if any) and has forwarded its final opinion on Part I to the Spanish Medicines Agency.

That this Committee has assessed part II of the application for authorisation of the trial in accordance with the provisions of Royal Decree 1090/2015 and Article 7 of Regulation (EU) 536/2014 and considers that:

- The procedure for obtaining informed consent (including the subject information sheets and informed consents mentioned in the heading), and the envisaged subject recruitment plan are adequate and meet the requirements for obtaining informed consent set out in Chapter II of Royal Decree 1090/2015. Versions in other languages of the above-mentioned approved documents may be produced, and it is the sponsor's responsibility to guarantee to the IRB/IEC that they are a faithful translation of the documents approved by the IRB/IEC.
- The compensation provided to the participants is adequate, as are the provisions for compensation for damages that the participant may suffer.
- The procedure foreseen for the handling of personal data is adequate.
- The future use of the biological samples obtained during the test is in accordance with the provisions of Royal Decree 1716/2011.
- The centers and investigators referred to in Annex II of this opinion are deemed suitable for carrying out the trial, considering the declarations of suitability made by the sponsor and the heads of the institutions concerned.

The Committee decided to issue a favourable opinion at the meeting of May 11th, 2018 (UM_ACTA Minutes).
That in said meeting the requirements established in the legislation in force - Royal Decree 1090/2015 - were met in order for the decision of the aforementioned Ethics Committee for Clinical Research with Medicines to be valid.

That the Ethics Committee of the Vall d'Hebron University Hospital, both in its composition and in its procedures, complies with the GCP standards (CPMP/ICH/135/95) and with the current

legislation regulating its operation. The composition of the Ethics Committee for Clinical Research with Medicines of the Vall d'Hebron University Hospital is the one indicated in annex I, taking into account that in the event that any member participates in the trial or declares any conflict of interest, he or she will be absent during the evaluation.

Sign in Barcelona, May 11th, 2018

Signed Mrs. Mireia Navarro

Annex I

ETHICS COMMITTEE COMPOSITION

President	Gallego Melcón, Soledad. Médico
Vice President	Segarra Sarries, Joan. Abogado
Secretary	Navarro Sebastián, Mireia. Química
Members	Armadans Gil, Lluís. Médico
	Azpiroz Vidaur, Fernando. Médico
	Balasso, Valentina. Médico
	Cucurull Folgera, Esther. Farmacóloga
	De Torres Ramírez, Inés M. Médico
	Fernández Liz, Eladio. Farmacéutico de Atención Primaria
	Fuentes Camps, Imma. Farmacóloga
	Gálvez Hernando, Gloria María. Diplomada Enfermería, Unidad Atención al Paciente
	Guardia Massó, Jaume. Médico
	Iavecchia, María Luján. Farmacóloga
	Joshi Jubert, Nayana. Médico
	Martínez Muñoz, Montserrat. Diplomada Enfermería,
	Hortal Ibarra, Juan Carlos. Profesor Universidad de Derecho
	Rodríguez Gallego, Alexis. Farmacólogo
	Sánchez Raya, Judith. Médico
	Solé Orsola, Marta. Diplomada Enfermería
	Suñé Martín, Pilar. Farmacéutica Hospital
	Vargas Blasco, Víctor, Médico

Vall d'Hebron
Hospital

Annex II

MAIN PARTICIPATING CENTRES AND RESEARCHERS IN SPAIN

Code : HEP101 EudraCT Number : 2016-001177-32
Title : Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure.

SPONSOR: Promethera Biosciences
UPDATE DATE ANNEX II: May 11th, 2018

Bellvitge University Hospital by Dr. José Castellote Alonso as Principal Investigator
Hospital de la Santa Creu i Sant Pau by Dr. German Soriano Pastor as Principal Investigator
Vall d'Hebron University Hospital by Dr. Victor Vargas as Principal Investigator
Hospital University Ramón y Cajal by Dr. Agustín Albillos as Principal Investigator
Hospital de Sabadell by Dr. Jordi Sánchez Delgado as Principal Investigator
Reina Sofía Córdoba Regional Hospital Complex by Dr. José Montero Álvarez as Principal Investigator
Virgen del Rocío Regional Hospital Complex by Dr. Manuel Romero Gómez as Principal Investigator
Hospital University Polytechnic La Fe by Dr. Vanessa Hontangas as Principal Investigator

REFERENCE: MUH/CLIN/EC

SUBJECT : RESOLUTION OF THE REQUEST FOR AUTHORIZATION OF A CLINICAL TRIAL

RECIPIENT: Promethera Biosciences, Rue Granbonpré, 11, B-1435 Mont-Saint-Guibert (Belgium)

DATA OF REQUEST :

Request for authorization of the Clinical Trial with EudraCT Nº 2016-001177-32 and title Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure.

SPONSOR: Promethera Biosciences

Date valid request: 13/03/2018

After evaluation of the request for authorization of a clinical trial previously, it is considered to be compliant with the requirements indicated in Royal Decree 1090/2015, of December 4, which regulates clinical trials with medicines, the Ethics Committees of Research with Medicines and the Spanish Registry of Clinical Studies and other applicable legislation *.

For all the above, the Director of the Agency of Medicines and Healthcare Products in the exercise of his/her competences RESOLVES:

AUTHORIZE the requested clinical trial and UPDATE the qualification of the drug HepaStem, with PEI Number 14-112, as a product in the clinical research phase, with the indication: acute-on-chronic liver failure.

NOTES FOR THE APPLICANT:

For future submissions, the Applicant is advised to consider the following recommendations:

The Applicant provides results related to the proportion of liver cell types in LCS depending on the manufacturing site (Table 2.3.S-47). It would be more convenient if the Applicant uses the same cell markers for each kind of cells independently of the LCS manufacturing site.

Indoleamine-pyrrole 2,3-dioxygenase (IDO) secretion levels are expressed in epg/106 cellsf in tables 2.3.S-50 and 2.3.S-51, but in table 2.3.S-52, IDO levels are presented in eng/106 cellsf. The Applicant should be consistent with the quantification scale used.

The Figure 2.3.S-15 is incomplete; the graphic with TNF¿ production is not present in the dossier.

* Consolidated text of the Law on Guarantees and Rational Use of Medicines and Medical Products, approved by Royal Legislative Decree 1/2015, of July 24. Royal Decree 1275/2011, of September 16, which creates the State Agency "Spanish Agency for Medicines and Health Products" and approves its Statute".

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Against this Resolution, which puts an end to the administrative procedure, a Remedy of Appeal may be lodged with the Director of the Spanish Agency for Medicines and Health Products within a period of one month, in accordance with the provisions of articles 123 and 124 of the Law 39/2015, of October 1, of the Common Administrative Procedure of the Public Administrations, or to file a Contentious-Administrative Appeal before the Central Contentious-Administrative Court of Madrid, within a period of two months from the day after the receipt of this notification, in accordance with the provisions of the Law on the Contentious-Administrative Jurisdiction of July 13, 1998, and without prejudice to any other remedy that may be filed.

THE DIRECTOR OF THE SPANISH AGENCY OF MEDICINES AND SANITARY PRODUCTS

By Authorisation (Article 14.4 of the Statute of the Agency approved by Royal Decree 1275/2011, of September 16.)

(BOE No. 229, of September 23, 2011)

GENERAL SECRETARY

Signed by Francisco Javier Muñoz Aizpuru

Digitally signed by: Agencia Española de Medicamentos y Productos Sanitarios
Date of signature: 23/05/2018
Locator: CALXSQVD41

You can check the authenticity of the document in the AEMPS Web Locator application

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РЕПУБЛИКА БЪЛГАРИЯ
Изпълнителна агенция по лекарствата
REPUBLIC OF BULGARIA
Bulgarian Drug Agency

Logo of BDA

PERMISSION

Ref No. КИ-109-2-0026 / 14-06-2018

Pursuant to art. 119, para.1, it.1, in relation with to art. 109, para.2 of the Low for medicinal products in human medicine

PERMIT
THE CONDUCT OF THE CLINICAL TRIAL WITH A TITLE:

Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure

Protocol №: HEP101
EudraCT №: 2016-001177-32
Application and documentation with ref. N/N ИАЛ-15137/10.04.2018, ИАЛ-21099/ 17.05.2018
Sponsor: PROMETHERA BIOSCIENCES, BELGIUM
Legal representative in the territory of EC:-
Applicant: COMAC MEDICAL LTD

Name / code and pharmaceutical form of the investigational medicinal product (products):

1. HepaStem, suspension for injection, 10 – 50 x 10^6 cells/ml

Investigational sites and principal investigators:

- Clinic of gastroenterology, „University multiprofile hospital for active treatment "Tsaritsa Yoanna - ISUL""Ltd., Sofia, with a principal investigator assoc.prof Jordan Genov;
- Department of gastroenterology, Clinic of internal diseases, "Multiprofile hospital for active treatment "Sveta Anna" Sofia",Sofia, with a principal investigator Dr. Bojidar Tomov;
- Department of clinical gastroenterology with hepatology, Clinic of gastroenterology, „University multiprofile hospital for active treatment Dr. Georgi Stranski" Ltd., Pleven, with a principal investigator dr. Desislava Lyubomirova Pavlova;
- Department of internal diseases, "Multiprofile hospital for active treatment Medika Ruse" Ltd, Ruse, with a principal investigator Dr.Lyudmil Haralampiev.

Yours faithfully,

ASSOC. PROF. ASSENA STOIMENOVA, PhD
Executive Director

Signature: illegible
Round stamp of the Bulgarian Drug Agency

София 1303, ул. Дамян Груев № 8, тел.: (02) 8903 555, факс: (02) 8903434
8, Damyan Gruev Str., 1303, Sofia, Bulgaria, tel.: +359 2 8903555, fax: +359 2 8903434
e-mail: bda@bda.bg

Letterhead of the Ethics Committee for Multicenter Trials

Logo of Republic of Bulgaria

REPUBLIC OF BULGARIA
MINISTRY OF HEALTH
ETHICS COMMITTEE FOR MULTICENTER TRIALS

Ref. No. КЕМИ/СТ-0296/25.06.2018

O P I N I O N
of
the Ethics Committee for Multicenter Trials

Pursuant to Article 111, Par. (2), Subpar. 1 of the Law on Medicinal Products for Human Medicine during its meeting held on 19.06.2018, in the presence of the necessary quorum and in the absence of conflict of interest, the Ethics Committee for Multicenter Trials made a decision to grant a **favorable opinion** regarding the conduct of the clinical trial entitled:

"Multicenter Phase II Safety and Preliminary Efficacy Study of 2 dose regimens of HepaStem in Patients with Acute-on-Chronic Liver Failure"

Protocol code: HEP101
EudraCT: 2016-001177-32
Application and documentation with Inc. Ref. No.КЕМИ -1335/11.04.2018 / КЕМИ-2010/18.05.2018
Sponsor: Promethera Biosciences, Belgium
Applicant: Comac Medical Ltd.

Name / Code and Pharmaceutical Form of the Investigational Medicinal Product / Products:

HepaStem, suspension for injection, 10 – 50 million organisms/ ml million organisms/milliliter

Investigational Sites and Principal Investigators:

1.MHAT "Sveta Anna" Sofia",Sofia, Clinic of internal diseases, Department of gastroenterology, Sofia	1. Dr. Bojidar Tomov
2. "UMHAT Dr. Georgi Stranski" Ltd., Pleven, Department of clinical gastroenterology with hepatology, Clinic of gastroenterology;	2.Dr. Desislava Lyuboslavova Pavlova
3. „UMHAT Tsaritsa Yoanna - ISUL""Ltd., Sofia, Clinic of gastroenterology	3. Dr. Jordan Genov
4., "MHAT Medika Ruse" Ltd, Ruse, Department of internal diseases	4. Dr.Lyudmil Haralampiev

Conditions for Exercise of Ongoing Supervision during the Conduct of the Clinical Trial:

1. After the commencement of the clinical trial (enrollment of the first patient on the territory of the Republic of Bulgaria), the Sponsor shall be obligated to notify the Committee in writing. The notification must include information about the title and code of the trial, the Sponsor, the

date of enrollment and code of the first patient, and the investigational site where the first patient is enrolled. The notification must take place within 15 days of enrollment of the first patient in the site.

2. Trial status reports must be provided once a year. For trials that last less than one year, a report must be provided after the elapse of half of the term of the trial.

The Ethics Committee for Multicenter Trials operates according to the rules of Good Clinical Practice.

PROF.DR.TODOR POPOV
CHAIRMAN: signature elligible

Round stamp
REPUBLIC OF BULGARIA
MINISTRY OF HEALTH
SOFIA