TITLE:

Safety and preliminary efficacy trial of Heterologous Human Adult Liver-derived Progenitor Cells for treating Urea cycle disorders and Crigler-Najjar syndrome

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ABSTRACT :

BACKGROUND: Regenerative medicine using stem cell technology is an emerging therapy, addressing congenital and acquired diseases. Heterologous Human Adult Liver-derived Progenitor Cells (HHALPCs) have the potency to metabolise ammonium and conjugate bilirubin. Preclinical studies have shown the safety *in vitro* and in animal models.

AIM: We report the safety and preliminary efficacy results of a multicenter phase I/II trial using HHALPCs in pediatric patients with urea cycles disorders (UCD's) or Crigler-Najjar (CN) syndrome

METHODS: 14 urea cycle disorder patients and 6 Crigler-Najjar patients were included. Patients were divided in three weight cohort, and three dose levels. A dose escalation from the lowest to the highest dose was applied for the first three patients enrolled in each weight cohort.

RESULTS: The safety profile observed was in line with the profile expected for this new cell therapy, the infusion procedure, the concomitant medications, the age group and the underlying disease. Partial left portal vein permanent thrombosis occurred in one patient. Preliminary efficacy data included *de novo* urea synthesis as demonstrated by the [^{13}C] ureagenesis test at 6 months follow-up versus baseline, engraftment of cells in 3 evaluable patients, and a decrease of bilirubin under similar phototherapy condition in 2 Crigler-Najjar patients.

CONCLUSION: This study opens the way for a confirmatory study in a homogeneous cohort of patients with a defined treatment dose.

Take-home message: This study reports general safety of Human Adult Liver-derived Progenitor Cells infusion in patients with severe metabolic diseases. Confirmation of efficacy will be addressed in a homogenous cohort of patients with a defined treatment dosis.

INTRODUCTION

Urea cycle disorders (UCDs) and Crigler-Najjar (CN) syndrome are inherited metabolic diseases associated with significant medical complications, imposing heavy burdens on patients and families.

UCDs result from deficient/absent activity of one of the six enzymes of the urea cycle, an endogenous metabolic pathway involved in ammonia elimination, including carbamoylphosphate synthetase 1 deficiency (CPS1D; OMIM #237300), ornithine transcarbamylase deficiency (OTCD; OMIM #311250), argininosuccinate synthetase deficiency (ASSD; OMIM #215700), argininosuccinate lyase deficiency (ASLD; OMIM #207900), arginase 1 deficiency (ARG1D; OMIM#207800), and N-acetyl glutamate synthase deficiency (NAGSD; OMIM #237310). These deficiencies are typically characterized by hyperammonemia and the accumulation of intermediary metabolites like glutamine. Brain damage is common, severe forms being potentially lethal (Ah Mew et al 2013; Brassier et al 2015). UCDs are orphan diseases observed in 1 in 35,000 live births (Summar et al 2013), with OTCD as the most common (Batshaw et al 2014; Brassier et al 2015).

Low protein diet is a cornerstone of UCD therapy, minimizing the nitrogen load on the urea cycle, yet potentially associated with complications like growth retardation (Haberle et al 2012; Batshaw et al 2014). UCD patients require L-arginine or L-citrulline supplementation, except in ARG1D. Nitrogen scavengers like sodium benzoate and sodium phenylbutyrate are also widely used to increase waste nitrogen excretion (Haberle et al 2012; Diaz et al 2013), but these treatments do not modify the metabolic defect itself and sodium phenylbutyrate may contribute to branched-chain aminoacid deficiencies (Burrage et al 2014). While liver transplantation corrects the metabolic defect regarding ureagenesis, this intervention is reserved for severe phenotypes, with transplantable livers being in short supply.

Crigler-Najjar syndrome (CN) is a very rare congenital disorder affecting bilirubin metabolism, resulting in severe unconjugated hyperbilirubinemia due to a hepatic deficit of uridine diphosphate (UDP) glucuronosyltransferase (UGT1A1) activity. The annual incidence at birth is 1/1,000,000, and clinical symptoms manifest from the first days of life. Two types have been described: CN1 and CN2. CN1 is characterized by complete enzyme deficiency, and CN2 by only partial deficit, both associated with a risk of brain injury (kernicterus), particularly during intercurrent illness or following sudden bilirubin level increases (Jansen 1999; Kaneko et al 2000).

CN1 management relies on phototherapy for 10-12 hours/day (Bosma 2003), with orthotopic liver transplantation as the only curative treatment (Sokal et al 1995). Severe CN2 requires daily administration of phenobarbital and phototherapy.

The available treatments for UCD and CN are, at best, palliative. Healthy-cell infusion could offer a more definitive therapy aimed at restoring metabolic competency to the recipient liver. To this end, both disorders have been treated experimentally with hepatocyte infusions (Fox et al 1998; Stephenne et al 2005; Stephenne et al 2006; Lysy et al 2008; Jorns et al 2012). The rationale is to confer sufficient metabolic competence to the liver for attenuating the clinical course until allogenic hepatic transplantation becomes feasible (Meyburg et al 2009).

Stem-cell technology was applied in selected patients under the hospital exemption regulation, confirming the feasibility and engraftment potential, while demonstrating the liver's biodistribution of cells infused *via* the portal route (Sokal et al 2013; Defresne et al 2014).

This report summarizes the Phase I/II trial results using Heterologous Human Adult Liver-derived Progenitor Cells (HHALPCs) (Najimi et al 2007; Khuu et al 2011; Khuu et al 2013) (Promethera HepaStem, Promethera Biosciences, Mont St. Guibert, Belgium) for UCD and CN treatments.

MATERIALS AND METHODS

Health authorities and ethical review

The study was approved by the competent authorities and ethics committees (EC) of each participating country (Belgium, France, United Kingdom, Italy, and Israel). The protocol was reviewed by the pediatric committee of the European Medicines Agency (EMA) and conducted in accordance with the 2000 revised Helsinki Declaration. Written informed consent was obtained from all patient guardians or, when appropriate, the patients themselves. The study data was regularly reviewed by a safety monitoring board.

Study population

Eligible pediatric patients had a confirmed diagnosis of CN or one of the following UCDs: CPSID, OTCD, ASSD, ASLD, ARGD, NAGSD; seriously impaired quality of life or severe disease despite maximal conservative treatment, rendering them a candidate for liver transplantation or alternatives. The main exclusion criteria were clinical or radiological evidence of fibrosis or cirrhosis, previous portal vein thrombosis, or persistent impairment to anterograde portal blood flow.

Study design

This partially-randomized, multicenter, open-label, dose-escalation Phase I/II study sought to include 21 pediatric patients suffering from UCD or CN. Patients were divided into three cohorts by body weight: Cohort 1: >20Kg, Cohort 2: ≥10-20Kg, and Cohort 3: <10Kg (Tables 1A and 1B). Three dose levels were investigated: low ($12.5x10^6$ cells/Kg), intermediate ($50x10^6$ cells/Kg), and high ($200x10^6$ cells/Kg) ($4x10^9$ maximum total cell count). Dose escalation from the lowest to highest dose was applied to the first three patients enrolled in

each cohort, with randomized treatment (intermediate/high dose) for Patient 4 onwards in Cohorts 1 and 2.

Product formulation and administration

HepaStem was granted the status of advanced medicinal product (ATMP) and designated as an orphan drug by EMA.

HepaStem, a mesenchymal progenitor cell, is derived from healthy human livers of donor origin (Najimi et al 2007). The cells were purified and expanded *in vitro* in five successive passages, then harvested, cryopreserved in CryoStor-10 (10% dimethyl sulfoxide [DMSO]), and stored in liquid nitrogen.

Before use, the cells were thawed and washed in albumin solution to remove the DMSO, then formulated in the aseptic environment at Promethera Biosciences GMP facility or in a fully-closed GMP-compliant formulation system based on Biosafe's SEPAX 2 device (Biosafe SA, Switzerland), installed in a mobile GMP-approved formulation facility or dedicated laboratory. The drug product (DP) was presented in a 50mL-bag containing ~250x10^6 HHALPC 0.084% sodium bicarbonate, 5% human albumin, and 500 IU heparin. Cells originating from 3 donors were used for the study, each patient received cells originating from a single liver donor. The cell viability at the time of infusion was 60-92%.

HepaStem was infused intravenously *via* a percutaneous transhepatic portal catheter, inserted under general anesthesia by direct transhepatic puncture of the right/left portal vein and under ultrasound or radiologic guidance, with the tip pushed in retrograde direction to the main portal vein at the splenomesenteric confluent.

HepaStem was administered as one cycle of one or several infusions. For each infusion, 50-100mL (250-500x10^6 cells) of HepaStem were administered at a flow rate of 0.5-2 mL/min, with 2-6 hours or a night in-between.

HepaStem was given in addition to each individual patient's treatment.

Concomitant therapy

HepaStem infusions were performed under moderate anticoagulation with bivalirudin (Angiox®, The Medicines Company UK Ltd) to prevent coagulation cascade activation (Stephenne et al 2012). 1.75mg/Kg/h was administered 15 min before cell infusion, 1.75mg/Kg/h during, then 0.25mg/Kg/h until the next infusion or for 30 min following the last infusion of the day. To monitor the coagulation cascade, the activated clotting time was measured on fresh whole blood and maintained within the range of 200-350 seconds (normal values: <100 seconds) (Stephenne et al 2012). Immunosuppressive treatment was given to all patients. Basiliximab (Simulect®, Novartis) was administered on infusion Days 1 and 4 (5mg/day for <15Kg body weight, 10mg for 15-35Kg, 20mg for >35Kg). Tacrolimus (Prograf® or Modigraf®, Astellas Pharma) was initiated following transhepatic catheter insertion, continued throughout the study aiming at reaching a blood level of 10±2ng/mL in post-infusion Month 1, 8±2ng/ml Week 6 to Months 2-3, and 6±2ng/mL thereafter. Additionally, one single 2mg/Kg methylprednisolone dose (Solumedrol®, Pfizer) was given in the morning before each infusion as a prophylactic measure against allergic or inflammatory reactions.

Safety evaluation

Safety evaluation was based on adverse event (AE) and serious adverse event (SAE) reporting with assessment of relation to cell infusion and concomitant treatments. Abnormal clinical and laboratory results considered clinically significant were reported as AEs. Vital signs, physical examination, common laboratory tests, anti-human leukocyte antigen (HLA) antibodies, D-dimer evaluation, liver echography and Doppler findings, along with portal vein flow and pressure values were recorded. HLA antibody levels were considered significant when mean fluorescence index (MFI) positivity cutoff was >1000 (Reed et al 2013).

Liver biopsy was performed at baseline, 6 months, and 12 months to evaluate liver histology (Tuchman et al 2008). METAVIR score was used to evaluate fibrosis (Bedossa and Poynard 1996). Analyses were performed in local laboratories according to local practices, a second central review being also performed.

Preliminary efficacy evaluation

Although this Phase I/II study was primarily designed for safety and dose-finding assessments, preliminary efficacy data like laboratory and clinical parameters was also recorded. In UCD patients, blood ammonia and plasma amino-acid profiles (+/- urinary orotic acid), metabolic decompensations (hyperammonemia episodes at hospital), natural protein and aminoacid supplement intake, and nitrogen scavenger doses were collected. Additionally, *in vivo* ureagenesis was assessed using stable non-radioactive isotopes to evaluate actual urea cycle activity, while incorporating ^{13}C into the urea from ^{13}C-labeled precursor (Tuchman et al 2008; Yudkoff et al 2010; Ah Mew et al 2014). UCD patients ingested 0.33mmol/Kg (27mg/kg) sodium [1-^{13}C] acetate dissolved in 60mL of water. Blood was taken before labeled precursor ingestion, then every 30 minutes for 2 hours. To integrate plasma [^{13}C] urea concentrations measured over 2 hours, the plasma [^{13}C] urea area under the curve (AUC)-120 min was calculated (µmol*min/L). Tests were performed at baseline and at 3, 6, and 12 months and samples sent to a central laboratory (Children's Hospital of Philadelphia, USA).

HepaStem engraftment was assessed on liver biopsy samples taken at baseline, 6, and 12 months post-cell infusion. Immunohistochemistry was used to detect target enzyme activity (*Institut de Recherche Expérimentale et Clinique, PEDI, Université Catholique de Louvain*, Brussels, Belgium), and analysed in UCD patients in a central laboratory (*Laboratoire de biochimie métabolique, Hôpital Universitaire Necker-Enfants malades*, France) (Schimke 1962; Nuzum 1976; O'Brien and Barr 1981; Rabier et al 1989). Additionally, fluorescence *in*

situ hybridization (FISH) was used to detect the Y chromosome from male donor cells in female recipients.

In CN patients, total and indirect serum bilirubin levels were monitored, along with phototherapy duration and hyperbilirubinemia episodes (*i.e.*, sudden increase of patients' individual level requiring intensified phototherapy in hospital).

Statistical analyses consisted of descriptive statistics performed on the total safety population. For the ureagenesis test, a specific mixed-effect ANCOVA on ^{13}C-urea AUC was added for exploratory purposes. SAS software was used for analyses.

RESULTS

Study population and exposure to HepaStem

In total, 21 patients were screened and 20 treated with HepaStem: 14 UCD and six CN; Cohort 1 (patients >20Kg) included eight UCD and two CN patients; Cohort 2 (patients ≥10-20Kg): three UCD and four CN patients; Cohort 3 (patients <10Kg): three UCD patients (Tables 1A and 1B).

Two UCD and two CN patients were assigned to low dose ($12.5x10^6$ cells/Kg), five UCD and one CN patients to medium dose ($50x10^6$ cells/Kg), and seven UCD and three CN patients to high dose ($200x10^6$ cells/Kg) (Table 1A and 1B). Infusions were administered over 1 to 4 days. The high dose could not be fully administered in five patients due to catheter displacement (n=3), transfusion reaction (n=1), and D-dimer values >20 000ng/mL (nL <500) (n=1).

Safety

During hospitalization for HepaStem administration and the following days post-infusion, mild AEs were reported, including laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation agents administered during HepaStem infusion were well tolerated.

SAEs related to HepaStem administration or catheter placement occurred in seven patients (Table 2). Symptomatic metabolic decompensations occured in five UCD patients, one between infusions and four within Days 2-4 post-infusion. These five patients were three OTCD female adolescents, one 10-year-old ASLD patient, and one 7-year-old ARGD patient who also exhibited transient transaminase increases. Treated at hospital, these events resolved in a few days. These patients did not receive specific intensified preventive treatment during infusion (*i.e.*, intravenous glucose, arginine, and nitrogen scavengers, transiently reduced-protein diet). Those who did presented no metabolic decompensation. One of the three cited OTCD adolescent female patients also developed a left portal vein thrombosis. This patient had received the number of cells per day (total of 3.5 billion cells in five infusions over two days). Another UCD patient, an adolescent OTCD male, developed intraluminal non-occlusive parietal thrombus of the main portal vein after removing the transhepatic catheter that had remained in place for five days. Both thrombotic events were treated using low-molecular-weight heparin (LMWH). The first thrombus was not resolved by the Month 12 follow-up visit, with persistent left portal vein flow interruption, yet normal liver functional parameters (liver echography, liver enzymes, and bilirubin). The second event fully resolved within one month.

One CN patient showing signs of a transfusion-like reaction was treated using methylprednisolone. A week later, infusions were restarted, and a similar event occurred. The condition resolved after stopping the infusion.

Beyond the infusion period, in both disease groups, the AEs were in line with common AEs observed as correlated to the age and morbidity of the studied population. Reported infections were primarily upper respiratory tract and gastrointestinal infections. Five UCD patients displayed one to three hyperammonemia episodes that were treated at the hospital. In addition, three female OTCD adolescent patients suffered from four to seven episodes each, with a context of non-compliance to supportive treatment in one case. Among the CN patients, one

CN1 developed repeated hyperbilirubinemia episodes between the first and third month post-HepaStem administration, possibly caused by an inadequate phototherapy device regarding his age/size. Two patients developed skin depigmentation areas on the limbs after Month 12 follow-up visit, one diagnosed with mycosis fungoides based on histology analysis, although neither molecular rearrangement nor genetic abnormalities were revealed on tissue biopsy. As the combined effect of tacrolimus and phototherapy was the suspected cause, tacrolimus administration was stopped. In both cases, the events resolved without sequelae.

Two patients developed donor-specific anti-HLA class I antibodies as detected at Month 6 follow-up visit, but had disappeared at Month 12 follow-up visit in one of them.

Efficacy in UCD patients

All UCD patients underwent a restricted natural protein diet, among which eight patients received aminoacid supplements. Nine received a total protein dose (natural protein and amino acid supplements) below the World Health Organization (WHO) "safe level" for protein intake (WHO Technical Report Series 2007). Three months preceding cell infusion, the median natural protein intake was 0.64g/Kg/day (range: 0.33-1.55g/kg/day), and the median total protein 1.0g/Kg/day (range: 0.6-2.0g/kg/day), corresponding to 92% (range: 70-200%) of the WHO "safe level". In the final three months, the median natural protein intake remained unchanged at 0.58g/Kg/day (0.20-1.16mg/kg/day), as did the total intake (median: 0.95g/Kg/day; range: 0.3-1.9g/Kg/day), corresponding to 84% (range: 35-165%) of the WHO "safe level".

All patients received at least one ammonium scavenger drug, with half receiving both sodium benzoate and sodium phenylbutyrate. Overall, the drug doses per kg were stable. In those treated with ammonium scavengers for three months before cell infusion, the median dose of sodium benzoate was 212mg/Kg/day (range: 104-449mg/Kg/day), and that of sodium phenylbutyrate 275mg/Kg/day (range: 99-543mg/kg/day). During the last three study months,

the median sodium benzoate dose was 208mg/Kg/day (range: 115-419mg/kg/day), and that of sodium phenylbutyrate 231mg/Kg/day (range: 81-419mg/kg/day).

Individual ammonia profiles revealed between-patient variability, fluctuating primarily below 80µmol/L with random peaks above 100µmol/L up to 411µmol/L. The median ammonia value at baseline was 49 µmol/L (min.: 19µmol/L; max.: 144µmol/L), and at last follow-up visit 53µmol/L (min.: 5µmol/L; max.: 125µmol/L).

During the study, glutamine values revealed individual and inter-patient variability, yet stabilizing over time, with values varying between 800 (upper normal range for healthy individuals) and 1000µmol/L (clinically-acceptable threshold for UCD patients). The median glutamine value at baseline was 915µmol/L (min.: 361µmol/L; max.: 1496µmol/L), and at last follow-up visit 1082µmol/L (min.: 267µmol/L; max.: 1330µmol/L).

Patients exhibited variable [^{13}C] urea AUC-120 values at baseline, nine exhibiting values between 4 and 51µmol*min/L, clearly lower than healthy subjects (mean: 256 µmol*min/L evaluated outside of this study), and two exhibiting 156 and 181µmol*min/L, respectively. Unexpectedly, one patient had a high baseline value of 301µmol*min/L, as yet unexplained (Table 3). Given the high variability between baseline results and the fact that the values were not normally distributed, we conducted ANCOVA with baseline [^{13}C] urea AUC-120 as covariate (Figure 1). For the entire UCD group, this analysis revealed a statistically significant increase in the [^{13}C] urea AUC-120 geometric mean (GM) at the Month 6 follow-up visit *versus* baseline (GM ratio: 1.90, 95% confidence interval [CI]: lower limit [LL]: 1.22, upper limit [UL]: 2.97; p-value: 0.007). At Month 3 follow-up visit, all patients had positive ratios, except one. This resulted in a non-statistically significant change from baseline due to the single patient's unexplained very high baseline value. At Month 12 follow-up visit, no significant change from baseline was observed (Table 3).

In vitro tests on random liver biopsy samples revealed low enzymatic activity compared to healthy subjects (<1 to ~10% in OTCD, ~20-30% in the other disorders). Variations observed between time points were within the method's variability.

Efficacy in CN patients

All CN patients were treated with prolonged (10-12h) daily phototherapy. Data at Baseline visit revealed high variability between patients, with individual values of 203.5 to 381.3µmol/L for a median of 312.95µmol/L.

All CN patients were referred to the principal investigator for the procedure, using other phototherapy devices while travelling out of their country (n=5/6). Moreover, some patients' phototherapy devices were changed during the study, as their original devices were considered inappropriate (Figure 2A). Nevertheless, for two patients (low-dose), a ~20% decrease in total serum bilirubin levels could be attributed to HepaStem by analysing periods where each patient used the same phototherapy devices (Figure 2A and 2B). For a third patient, a transient effect was observed. No improvement in total serum bilirubin levels was observed in the three other CN patients.

Evaluation of cell engraftment

Random percutaneous liver biopsies were performed at baseline, Month 6, and Month 12 follow-up visits. Of the three evaluable UCD patients with negative immunohistochemistry for the missing enzyme at baseline, immunohistochemistry tests revealed OTC-positive cells at Month 6 in one OTCD patient, and arginase-positive cells in one arginase 1 D patient.

In one CN patient (female recipient of male cells, low dose), FISH performed on Month 12 follow-up liver biopsy for gender chromosomes revealed 78% male cells on 65 nuclei. Interestingly, this patient was one of the two exhibiting a 20% decrease in total serum bilirubin.

DISCUSSION

Stem cells are classified as advanced therapy medicinal products (ATMPs), their use in humans being subject to specific regulations. We report here the first clinical trial results using liver-derived progenitor cells to treat children with rare liver-based inborn errors of metabolism.

This Phase I/II study included UCD patients suffering from different enzymatic deficiencies exhibiting severe phenotypes, low residual enzyme activity, and low protein tolerance, requiring nitrogen scavengers yet still at risk of metabolic decompensation. This population represented only a fraction of the overall UCD population (Kolker et al 2015; Kolker et al 2015). Given that these patients' quality of life remains poor despite conventional therapy, innovative disease-modifying therapies are being developed (Sokal et al 2013; Sokal 2014). All included CN patients displayed elevated serum bilirubin levels, requiring constraining phototherapy impairing their quality of life.

This study was designed to evaluate the safety of the medicinal product and its administration procedure at different dose levels, the quantity of cells administered ranging from 0.25% to 4% of the total estimated liver cell mass (Wilson et al 2003; Sohlenius-Sternbeck 2006; Bianconi et al 2013). The safety profile observed corresponded to expectations for this new cell therapy, infusion procedure, concomitant medications, age group and underlying diseases, with safety data confirming the tolerability of HepaStem administered via portal vein infusion procedure, provided that precautionary treatment measures were in place, especially when considering UCD patients.

Higher HepaStem doses, associated with prolonged transhepatic intraportal catheterization, immobilization and hospitalization, appeared to increase the risk of adverse reactions, while lower doses given on one single day were very well tolerated. Younger patients with severe diseases displayed no increased risk of developing adverse reactions.

Prophylaxis of deep vein thrombosis remains a concern in all intravascular procedures and surgical procedures in general. In liver transplantation, the incidence of portal vein thrombosis was estimated at 10%, and deep vein thrombosis was shown to occur following bone marrow and hepatocyte transplantations (Baccarani et al 2005; Gerber et al 2008; Ueda et al 2008; Jensen et al 2013).

Our dose-finding study confirmed the risk of portal vein thrombus, which occurred in one UCD patient receiving a total infusion dose slightly exceeding the protocol recommendation. A second thrombus occurred post-infusion in a patient fitted with a catheter not specifically recommended for the perfusion (curled catheter) that remained *in situ* for five days. These two events emphasize the special attention required for selecting appropriate perfusion equipment, as well as the need to carefully monitor portal flow during infusion.

Metabolic decompensation was observed during HepaStem infusion in some UCD patients, yet not in children prophylactically placed under protective metabolic support, as recommended during intercurrent illnesses or medico-surgical procedures. No CN patient displayed decompensation during infusion.

Following the infusion period, the AE rate decreased, with most AEs involving episodes of metabolic decompensation and infections. UCD patients exhibited more AEs than CN patients, as expected for this fragile condition at risk of developing intercurrent illnesses or consecutive metabolic decompensations. The OTCD adolescent female patients exhibited the highest metabolic decompensation rates. Adolescence is a known at-risk period for chronic disease patients, due to both metabolic adjustments and decreased compliance (McGrady and Hommel 2013).

It was shown that liver-derived mesenchymal stem cells (MSCs) have a low immunogenic profile and exert in vitro a similar immunoregulatory effect as compared with bone marrow-derived MSCs (Sana et al 2014; Raicevic et al 2015). Detection of anti-HLA antibodies was

very limited in this study and their significance remains uncertain. Further studies should investigate any correlation between cells dosage, HLA mismatch and engraftment as reported in preclinical studies (Ankrum et al 2014; Isakova et al 2014).

Our study was also designed to explore the preliminary efficacy of HepaStem infusion, with the study groups including different diseases, weight groups, and cell dosages. Due to disease severities, long-term chronic supportive treatments were not modified, and HepaStem was given in addition to these treatments. Despite these limitations, clinical and functional preliminary efficacy of HepaStem infusions was observed. The urea ^{13}C ureagenesis test was used to evaluate the urea cycle functionality in UCD patients. This test, believed to be an independent measurement of the cycle avoiding interference from other parameters like treatment compliance (Ah Mew et al 2014), has been used to assess treatment efficacy in other studies (Tuchman et al 2008; Ah Mew et al 2010). Increased ureagenesis was observed in most UCD patients following HepaStem infusion, with overall improvement statistically demonstrated at Month 6 post-infusion. Yet further test validation in the context of a the disease-modifying setting appears required. For CN patients, a key lesson was the phototherapy's major impact on bilirubin values, as well as the quality of the lamps used, with a possible combined phototherapy-HepaStem effect noted in several patients. When evaluating comparable periods, the ~20% decrease in total serum bilirubin levels could be attributed to HepaStem in two patients.

We found encouraging data suggesting HepaStem integration in the liver. Immunohistochemistry of random biopsy samples for the deficient enzymes revealed cells present in two UCD patients at Month 6 post-infusion. In one female CN patient, FISH immunostaining revealed a cluster of 78% XY donor cells at Month 12 post-infusion. Cells were previously reported to be observed several months following hepatocyte transplantation

(Stephenne et al 2006), yet not so far in terms of stem cell therapy, this finding suggesting long-term benefits for patients.

In conclusion, this safety study confirms the tolerability of HepaStem in pediatric patients displaying inborn errors of liver metabolism. We present preliminary evidence of cell engraftment and *de novo* functional *in vivo* enzyme activity in the recipient liver, along with early indications of functional efficacy. This study paves the way for future investigations into alternative infusion procedures to confirm HepaStem efficacy in UCD and CN patients, and for targeting new indications.

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Table 1A Urea cycle disorder patient population and exposure to HepaStem

Patient n°	Disease	Onset	Gender	Age at baseline	Weight at baseline (kg)	Cohort	Assigned dose (x10^6 cells/kg)	Received dose (x10^6 cells/kg)	Infusion period
1	OTCD	Late	F	16 years	58.0	I	12.5	12.92	1 day 1 infusion
2	OTCD	Early	M	11 months	8.17	III		14.38	1 day 1 infusion
3	OTCD	Late	F	15 years	68.9	I	50	50.29	4 days 6 infusions
4	OTCD	Late	M	14 years	34.2	I		51.17	3 days 5 infusions
5	ASLD	Early	M	10 years	33.1	I		42.62	2 days 3 infusions
6	OTCD	Late	M	2.5 years	14.75	II		46.1	1 day 2 infusions
7	ASLD	Early	M	1 year	9.25	III		51.46	1 day 1 infusion
8	OTCD	Late	M	14 years	63.0	I	200	63.49*	3 days 10 infusions
9	OTCD	Late	F	15 years	56.5	I		70.47*	3 days 7 infusions
10	OTCD	Late	M	17 years	44.5	I		93.93*	4 days 9 infusions
11	ARGD	Early	F	7 years	21.2	I		135.38	3 days 5 infusions
12	CPSID	Early	F	7 years	19.5	II		146.94	4 days 7 infusions
13	CPSID	Early	F	4 years	15.0	II		187.90	3 days 6 infusions
14	OTCD	Early	M	1.5 months	3.2	III		195.85	1 day 2 infusions

* Maximum of 4 billion cells due to capping

OTCD: ornithine transcarbamylase deficiency; CPSID: carbamoyl phosphate synthase I deficiency; ARGD: arginase deficiency; ASLD: argininosuccinate lyase deficiency; F: female; M: male

Table 1B Crigler-Najjar patient population and exposure to HepaStem

Patient n°	Disease	Onset	Gender	Age at baseline	Weight at baseline (kg)	Cohort	Assigned dose ($x10^6$ cells/kg)	Received dose ($x10^6$ cells/kg)	Infusion period
15	CN Type I	Early	M	8 years	29.0	I	12.5	13.06	1 day 1 infusion
16	CN Type I	Late	F	4 years	13.5	II		12.59	1 day 1 infusion
17	CN Type I	Early	M	4 years	17.3	II	50	49.71	1 day 2 infusions
18	CN Type I	Early	F	6 years	20.9	I	200	96.83	8 days 4 infusions
19	CN Type II	Late	F	4 years	18.5	II		98.35	2 days 4 infusions
20	CN Type I	Early	F	3 years	15.02	II		61.54	1 day 2 infusions

Table 2 Serious adverse events (SAEs) reported during the HEP001 study

System organ classification and preferred terms	Patients	Number of SAEs during study periods			Total
		Infusion period + few days	Up to 6 months	6-12 months	
Hepatobiliary disorders					
Portal vein thrombosis	2 UCD: Patients 3; 10	2	-	-	2
Infections and infestations					
Gastroenteritis	4 UCD: Patients 1; 2; 14; 12 1 CN: Patient 20	-	1	5	6
Infection (enteral)	1 UCD: Patient 14	-	-	1	1
Laryngitis	1 CN: Patient 16	-	1	-	1
Nasopharyngitis	1 UCD: Patient 13	-	1	-	1
Parainfluenza virus infection	1 UCD: Patient 13	-	1	-	1
Rhinovirus infection	1 UCD: Patient 13	-	-	1	1
Viral infection	1 UCD: Patient 14	-	-	1	1
Injury, poisoning and procedural complications					
Transfusion reaction	1 CN: Patient 18	2	-	-	2
Gastrointestinal disorders					
Abdominal pain	1 UCD: Patient 3	-	-	1	1
Investigations					
Increased serum bilirubin	1 CN: Patient 17	-	4	-	4
Decreased coagulation factor	1 UCD: Patient 10	-	1	-	1
Decreased portal vein flow	1 UCD: Patient 3	1	-	-	1
Increased transaminases	1 UCD: Patient 11	1	-	-	1
Metabolism and nutrition disorders					
Hyperammonemia	4 UCD: Patients 2; 3; 8; 9	2	10	3	15
Metabolic disorder	6 UCD: Patients 1; 5; 11; 12; 13; 14	3	3	7	13
Benign, malignant, and unspecified neoplasms (including cysts and polyps)					
Mycosis fungoides	1 CN: Patient 17	-	-	1	1
Total		**7**	**26**	**20**	**53**

UCD: urea cycle disorder; CN: Crigler-Najjar syndrome

Table 3 **[^{13}C] urea AUC-120 individual data at baseline, 3, 6, and 12 months and ratio *versus* baseline**

Disease/ Patient n°	Dose	Cohort	Baseline AUC	3 months AUC	3 months Ratio 3m/BL	6 months AUC	6 months Ratio 6m/BL	12 months AUC	12 months Ratio 12m/BL
CPSID									
13	High	2	50.52	110.64	2.19	68.75	1.36	140.63	2.78
12	High	2	300.90	18.93	0.06	127.83	0.42	147.74	0.49
OTCD									
1	Low	1	39.81	41.01	1.3	81.05	2.04	30.51	0.77
3	Inter.	1	156.02	ND	ND	168.27	1.08	62.67	0.40
9	High	1	5.67	18.99	3.35	21.63	3.81	13.01	2.29
4	Inter.	1	4.25	7.88	1.86	7.79	1.83	3.48	0.82
8	High	1	25.70	41.79	1.63	ND	ND	54.38	2.12
10	High	1	36.83	44.84	1.22	33.17	0.90	31.41	0.85
6	Inter.	2	U	282.06	U	DO	DO	DO	DO
2	Low	3	26.66	66.57	2.50	189.68	7.12	37.83	1.38
14	High	3	ND	7.56	U	19.35	U	U	U
ASLD									
5	Inter.	1	14.56	20.87	1.43	35.31	2.42	34.35	2.36
7	Inter.	3	5.15	59.76	11.62	25.76	5.01	57.14	11.10
ARGD									
11	High	1	180.54	ND	ND	146.61	0.81	46.34	0.26

ARGD: arginase deficiency; ASLD: argininosuccinate lyase deficiency; AUC: area under the curve; BL: baseline; CPSID: carbamoyl phosphate synthase I deficiency; DO: drop-out; Inter: intermediate; ND: test not done; OTCD: ornithine transcarbamylase deficiency ; U: unknown (not calculated because one time-point missing)

Figure 1 Mixed-effect ANCOVA of plasma [^{13}C] urea AUC-120 change from baseline

Geometric mean ratios (GMR) of [^{13}C] urea area under the curve (AUC)-120 at Months 3, 6, and 12 follow-up visits were calculated while considering this covariate and comparing the geometric mean (GM) to baseline at Month 3, 6, and 12 follow-up visits. The results reveal a statistically significant increase in [^{13}C] urea AUC-120 GM at Month 6 *versus* baseline (GMR: 1:90, 95% confidence interval [CI]: lower limit [LL]: 1.22, upper limit [UL]: 2.97, p-value: 0.007)

Figure 2 Total bilirubin values at scheduled visits for CN patients

(A) Patient total bilirubin profiles at follow-up. Dotted lines: periods when the patients used a device differing from their home device (hospital device). Three patients received a new home phototherapy device, as indicated by the arrows. Patient 20 discontinued the study before completion.
(B) Individual total bilirubin profiles during the study for Patients 16 and 17. Dotted lines: changes in phototherapy devices