



# Phase I/II Trial of Liver-derived Mesenchymal Stem Cells in Pediatric Liver-based Metabolic Disorders: A Prospective, Open Label, Multicenter, Partially Randomized, Safety Study of One Cycle of Heterologous Human Adult Liver-derived Progenitor Cells (HepaStem) in Urea Cycle Disorders and Crigler-Najjar Syndrome Patients

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**Background.** Regenerative medicine using stem cell technology is an emerging field that is currently tested for inborn and acquired liver diseases. **Aim.** This phase I/II prospective, open label, multicenter, randomized trial aimed primarily at evaluating the safety of Heterologous Human Adult Liver-derived Progenitor Cells (HepaStem) in pediatric patients with urea cycle disorders (UCDs) or Crigler-Najjar (CN) syndrome 6 months posttransplantation. The secondary objective included the assessment of safety up to 12 months postinfusion and of preliminary efficacy. **Methods.** Fourteen patients with UCDs and 6 with CN syndrome were divided into 3 cohorts by body weight and intraportally infused with 3 doses of HepaStem. Clinical status, portal vein hemodynamics, morphology of the liver, de novo detection of circulating anti-human leukocyte antigen antibodies, and clinically significant adverse events (AEs) and serious adverse events to infusion were evaluated by using an intent-to-treat analysis. **Results.** The overall safety of HepaStem was confirmed. For the entire study period, patient-month incidence rate was 1.76 for the AEs and 0.21 for the serious adverse events, of which 38% occurred within 1 month postinfusion. There was a trend of higher events in UCD as compared with CN patients. Segmental left portal vein thrombosis occurred in 1 patient and intraluminal local transient thrombus in a second patient. The other AEs were in line with expectations for catheter placement, cell infusion, concomitant medications, age, and underlying diseases. **Conclusions.** This study led to European clinical trial authorization for a phase II study in a homogeneous patient cohort, with repeated infusions and intermediate doses.

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## INTRODUCTION

Inborn errors of liver metabolism (IELMs) are typically caused by a single-enzyme defect that alters hepatic metabolism, frequently in the neonatal period. Hepatic enzyme deficiencies may induce the damage of distant organs such as brain. Prominent examples are urea cycle defects or Crigler-Najjar (CN) syndrome. Current therapy for IELM is primarily supportive and involves dietary intervention, medications and, in unstable patients, orthotopic liver transplantation. IELMs are rare diseases, but collectively they represent approximately 10% to 50% of pediatric liver transplantation cases. The clinical utility of transplantation is limited by a shortage of donors, the risks of surgery, and the need for lifelong immunosuppression. Alternative therapies are based upon the administration of cells rather than transplantation of liver, an example being hepatocyte transfusion, which delivers otherwise deficient enzyme to the recipient liver.<sup>1-3</sup> Unfortunately, there is a dearth of qualified donor organs from which hepatocytes can be isolated. In contrast, a promising vehicle for restoration of hepatic enzyme activity is mesenchymal stem cells, which can be produced according to Good Manufacturing Practice standards in large amount and can be conveniently stored. These cells also can be differentiated into hepatocytes both in animal models and in vivo.<sup>4</sup>

Urea cycle disorders (UCDs) and CN syndrome are inherited metabolic diseases associated with significant medical complications, imposing heavy burdens on patients and submitting them to the risk of irreversible brain damage and death. UCDs can result from deficient/absent activity of 1 of the 5 (2 intramitochondrial, 3 cytosolic) enzymes of the urea cycle, a metabolic pathway involved in ammonia detoxification. These deficiencies cause hyperammonemia and accumulation of glutamine, a major alternative metabolic pathway derivative. UCDs are orphan diseases observed in 1 in 35 000 live births, ornithine transcarbamoylase deficiency (OTCD) being the most common.

CN syndrome is a very rare congenital disorder with an estimated incidence at birth of 1/1 000 000. It is characterized by a severe unconjugated hyperbilirubinemia due to hepatic uridine diphosphate glucuronosyltransferase deficiency. CN patients are managed with lifelong phototherapy (12 h/day) to control bilirubin levels via a conversion of unconjugated bilirubin into water-soluble bilirubin isomers subsequently excreted via the bile.

Heterologous Human Adult Liver-derived Progenitor Cells (HHALPCs, HepaStem) are mesenchymal cells obtained following collagenase digestion of the liver, with a preferential hepatocytic differentiation pattern.<sup>4,5</sup> HHALPCs have been safely and successfully administered under hospital exemption regulation in pediatric patients with IELM.<sup>5</sup> Such study has confirmed HHALPCs transplantation feasibility and showed their liver biodistribution following portal vein infusion.<sup>6</sup>

The aim of the current study was to evaluate the safety and dose escalation of HHALPCs at 6 and 12 months posttransplantation as well as their preliminary efficacy in a phase I/II prospective, open label, multicenter, partially randomized trial enrolling pediatric UCDs, and CN patients.

## MATERIALS AND METHODS

### Health Authorities and Ethical Review

The study protocol was reviewed and approved by the European Medicines Agency's (EMA) pediatric committee (EMA paediatric investigation plan number: EMEA-001155-PIP01-11). This multicenter trial involved 12 centers located in 5 countries and institutional review board approval was obtained by regulatory authorities and ethics committees from each participating country (Protocol numbers: 2011/04OCT/388, Commission d'Éthique Biomédicale, Hospitalo-Facultaire, Université Catholique de Louvain, Belgium); 12/LO/0564, UKHEP001 (Health

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The clinical trial was registered at the EU Clinical Trials Register at EudraCT. EudraCT identification number: 2011-004074-28.

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M.Y. is the head of the external laboratory who performed the ureagenesis test and participated in the manuscript revision.

B.D.V. and E.S. were responsible for the study design and protocol writing.

B.D.V., J.T., and E.S. were responsible for the study planning, literature search, data extraction and analysis, and manuscript writing. N.B., M.B., and M.N. were responsible for the manuscript writing.

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Research Authority, NRES Committee London, Hampstead, London), CPP 12/22 (Comité de Protection des Personnes Nord Ouest IV, Lille, France), Studio no 568/2012, Protocollo HEP001 (Comitato Etico, Ospedale Pediatrico Bambino Gesù, Roma, Italy); Helsinki Committee application number: 0524-12-RMB (Rambam Medical Center, Israel), Helsinki Committee application number: 0047-13-HMO (Hadassah Medical Organization, Israel); Helsinki Committee application number: 0129-13-RMC (Rabin Medical Center, Israel). The 3 centers from Israel had the approval number at Ministry of Health: HTA6499. The study was conducted in accordance with the International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP) E6 (R1). Written informed consent was obtained from parents and from patients whenever appropriate. The clinical trial was registered at the EU Clinical Trials Register at EudraCT under the identification number: 2011-004074-28. Data generated from the study were monitored by a safety monitoring board of independent experts.

### Enrollment Criteria

Pediatric patients with a confirmed diagnosis of UCD or CN by genetic mutation analysis were eligible for the study. General inclusion criteria included patient and/or legal representative providing written informed consent, negative pregnancy test for female subjects with childbearing potential, and patency of the portal vein and branches. Specific inclusion criteria for CN patients included diagnosis of CN poorly responsive to phenobarbital treatment. Specific inclusion criteria for UCD patients included a disease of such severity (eg, poor protein tolerance and recurrent hyperammonemic crises despite maximal conservative metabolic treatment) to warrant liver transplantation or alternatives. Exclusion criteria for both diseases included clinical or radiological evidence of liver fibrosis, cirrhosis, or portal vein thrombosis.

Due to the very low incidence of CN, an empiric minimum of 4 patients suffering from CN has to be recruited to allow the exploration of safety and tolerability of HepaStem under this condition.

T1 The characteristics of the 14 UCD and 6 CN patients treated with HepaStem are described in Tables 1A and 1B.

### Study Design

F1 This dose escalation phase I/II study sought to include  $21 \pm 2$  pediatric patients. Selected Patients were divided into 3 cohorts based on body weight (BW): cohort 1: BW > 20 kg, cohort 2: BW, 10-20 kg, and cohort 3: BW < 10 kg (Figure 1). Three HepaStem doses were investigated: low ( $12.5 \times 10^6$  cells/kg), intermediate ( $50 \times 10^6$  cells/kg), and high ( $200 \times 10^6$  cells/kg) dose (with a maximum of  $4 \times 10^9$  total cells infused). The range of doses originates from published clinical cases of patients transplanted with adult-derived human liver stem/progenitor cells (ADHLSs)<sup>7</sup> and with hepatocytes.<sup>1,3,8-10</sup> The dose escalation was performed in both intra and intercohorts (Figure 1). Dose allocation was randomized, starting from the lowest dose for the first 3 patients enrolled in each cohort. Treatment allocation (only intermediate and high doses) was randomized for the next patients (from patient 4 onwards) in cohorts 1 and 2. Cohort 3 consisted of 3 infants. Both

safety and tolerability of the cell infusion were appreciated via physical examination, adverse event (AE) assessments, and laboratory test changes. Potential efficacy included changes in metabolic status and the applied diets/supportive treatments.

### HepaStem Product

The generic name of HepaStem is HHALPC. The liver was disaggregated and a liver cell suspension was delivered by a Ministry of Health–approved liver tissue bank at Cliniques Universitaires St Luc, Brussels, Belgium. HepaStem is derived from the parenchymal fraction, expanded in vitro as previously described<sup>4</sup> and cryopreserved until use in a Good Manufacturing Practice–approved plant at Promethera Biosciences, Mont St Guibert, Belgium. HepaStem was granted with the advanced therapy medicinal product status by the EMA and designated as an orphan drug by the European commission and FDA.

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### Cell Infusion

HepaStem was administered as 1 cycle of one or several infusions, depending on the dose assigned and patient's BW. Each patient received cells derived from one single liver donor. HepaStem was infused via a percutaneous transhepatic portal catheter, inserted under general anesthesia by direct puncture of right/left portal vein under ultrasound or radiologic guidance. The tip was placed in the main portal vein at the splenomesenteric confluent. HepaStem infusions were performed under prophylactic bivalirudin anticoagulation (Angiox, The Medicines Company UK Ltd) to prevent coagulation cascade activation.<sup>11</sup> During infusion, the activated clotting time (ACT) was measured on fresh whole blood and maintained within 200 to 350 seconds (normal values: <100s).<sup>11</sup> Immunosuppression protocol was based on practice for liver transplantation and liver cell transplantation. Basiliximab (Simulect, Novartis) was administered IV on days 1 and 4 (5 mg/day for patients <15 kg BW, 10 mg for 15 to 35 kg, 20 mg for >35 kg). Tacrolimus (Prograf or Modigraf, Astellas Pharma) was initiated following transhepatic catheter insertion, to reach blood levels of  $10 \pm 2$  ng/mL during the first month,  $8 \pm 2$  ng/mL during months 2 to 3, and  $6 \pm 2$  ng/mL thereafter. Additionally, a single 2 mg/kg methylprednisolone dose (Solumedrol, Pfizer) was given before each infusion.<sup>12-14</sup> A dose escalation with a 16-fold increase between the lowest and the highest doses was proposed to increase the discriminatory power to perceive a possible difference in safety and preliminary efficacy.

### Primary and Secondary Endpoints

According to ICH Guideline for Good Clinical Practice (ICH E6 (R1):1.2), the frequency, nature, and severity of AEs and severe adverse events (SAEs) related to cell infusion were considered as primary endpoints up to 6 months and secondary up to 12 months.<sup>15</sup> Vital signs, physical examinations, clinical laboratory tests (liver and renal function, hematology, coagulation), anti-HLA and auto-immune serum antibody levels (anti-LKM, anti-SMA, and antinuclear antibodies) [mean fluorescence index > 1500<sup>16</sup>], portal vein pressure, echography, and Doppler exam of the liver were deeply evaluated to appraise the

**TABLE 1A.**  
Urea cycle disorder patient population and HepaStem transplantation conditions

Patient no	Disease	Onset	Gender	Genetic mutation	Age at baseline	Weight at baseline (kg)	Cohort	Assigned dose ( $\times 10^6$ cells/kg)	Total dose received ( $\times 10^6$ cells)	Received dose ( $\times 10^6$ cells/kg)	% Cells received /assigned	Infusion period
1	OTCD	Late	F	het c.540 + TG > T (nonfunctional)	16 y	58.0	I	12.5	765	13	106	1 day, 1 infusion
2	OTCD	Early	M	hem c.767 G > T (G256V)	11 mo	8.17	III		115	14	113	1 day, 1 infusion
3	OTCD	Late	F	het c.140DELA	15 y	68.9	I	50	3470	50	101	4 days, 6 infusions
4	OTCD	Late	M	hem c.524 A > G (N175G)	14 y	34.2	I		1750	51	102	3 days, 5 infusions
5	ASLD	Early	M	het c.719-2 A > G; c.857A > G (Q286R)	10 y	33.1	I		1415	43	85	2 days, 3 infusions
6	OTCD	Late	M	hem c.829 C > T (R277W)	2.5 y	14.75	II		680	46	92	1 day, 2 infusions
7	ASLD	Early	M	hom c.857 A > G (Q286R)	1 y	9.25	III		510	55	110	1 day, 1 infusion
8	OTCD	Late	M	hem (N198K)	14 y	63.0	I	200	4000	63	100 <sup>a</sup>	3 days, 10 infusions
9	OTCD	Late	F	het c.179 T > C (L179P)	15 y	56.5	I		4010	71	100 <sup>a</sup>	3 days, 7 infusions
10	OTCD	Late	M	hem c.386 G > A (R129H)	17 y	44.5	I		4180	94	105 <sup>a</sup>	4 days, 9 infusions
11	ARGD	Early	F	Exon 2 deletion	7 y	21.2	I		2870	135	72 <sup>b</sup>	3 days, 5 infusions
12	CPSID	Early	F	het c.849DELA (FS298X); c.2339G > A (R780H)	7 y	19.5	II		2645	136	68 <sup>c</sup>	4 days, 7 infusions
13	CPSID	Early	F	het c.2549 G > A (R850H); c.3520 C > T (R1174X)	4 y	15.0	II		2950	197	98	3 days, 6 infusions
14	OTCD	Early	M	hem c.958C > T (R320X)	1.5 mo	3.2	III		660	206	103	1 day, 2 infusions

<sup>a</sup>According to protocol, a maximum of 4 billion cells was administered in total.

<sup>b</sup>Full dose not received due to adverse event.

<sup>c</sup>Full dose not received due to catheter issues.

OTCD, ornithine transcarbamylase deficiency; CPSID, carbamoyl phosphate synthase I deficiency; ARGD, arginase deficiency; ASLD, argininosuccinate lyase deficiency; F, female; M, male; het, heterozygous; hem, hemizygous; hom, homozygous.

**TABLE 1B.**  
Crigler-Najjar patient population and HepaStem transplantation conditions

Patient no	Disease	Onset	Gender	Genetic mutation	Age at baseline (y)	Weight at baseline (kg)	Cohort	Assigned dose ( $\times 10^6$ cells/kg)	Total dose received ( $\times 10^6$ cells)	Received dose ( $\times 10^6$ cells/kg)	% Cells received/assigned	Infusion period
15	Type I	Early	M	hom c.1021 C>T (R341X)	8	29.0	I	12.5	380	13	105	1 day, 1 infusion
16		Late	F	het c.609_632 DEL (H203_K211DELINSQ); c. PROMOTOR [TA] 7, c.380G>C; (C127S)	4	13.5	II		170	13	101	1 day, 1 infusion
17		Early	M	hom c.A(TA)7TAA; c.530G>A (C176V)	4	17.3	II	50	845	49	98	1 day, 2 infusions
18		Early	F	hom c.A(TA)7TAA; c.-3279T>G; c.722_723DELAG (E241G FSX16)	6	20.9	I	200	1985	95	50 <sup>a</sup>	8 days, 4 infusions
19	Type II	Late	F	het c.-3279T>G; c.1198A>G; c.1220DELA	4	18.5	II		1790	97	48 <sup>b</sup>	2 days, 4 infusions
20	Type I	Early	F	hom c1220DELA (X)	3	15.02	II		1000	67	33 <sup>b</sup>	1 day, 2 infusions

<sup>a</sup>Full dose not received due to adverse event.

<sup>b</sup>Full dose not received due to catheter issues.  
F, female; M, male.

safety and tolerability of HepaStem as well as concomitant treatments. The monitoring of the morphology of the liver of the patients was also considered as a part of the safety assessment. Liver biopsy was performed before cell infusion and at 6 and 12 months postinfusion. Central histological review was organized blindly by 2 pathologists. METAVIR score was used to evaluate fibrosis.<sup>17</sup> The severity assessment for an AE/SAE was completed using the NCI Common Terminology Criteria for Adverse Events (CTCAE, version 4.0). Secondary efficacy endpoints included exploration of HepaStem efficacy from baseline up to 12 months postinfusion as well as its potential engraftment in recipient transplanted patients. Details on the assays performed for each indication are provided in the Supplementary Materials and Methods (SDC, <http://links.lww.com/TP/B674>).

### Statistical Analysis

Statistical analyses comprised descriptive statistics using SAS software. For continuous variables, descriptive statistics consisted of number of subjects, mean, median, SD, minimum, and maximum. Categorical endpoints were summarized using number of subjects, frequency, and percentages. In view of the explorative nature of the study, all analyses were performed on an intent-to-treat basis on the Total Safety Population. This population included all patients having received at least one infusion of HepaStem. For the ureagenesis test, a specific mixed-effect ANCOVA on <sup>13</sup>C-urea area under the curve (AUC) was used for exploratory purposes.

## RESULTS

### Safety of HepaStem Infusion

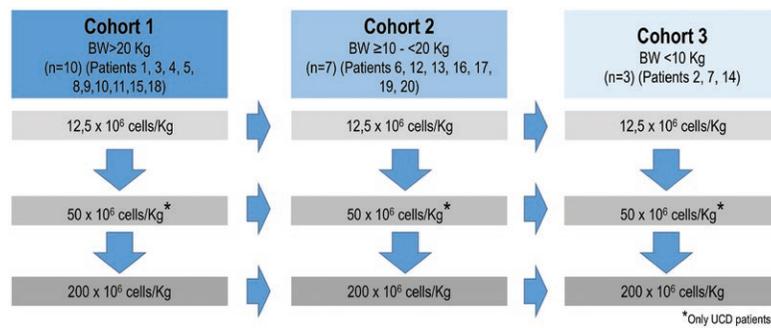
Infusions were administered over 1 to 4 days. Five patients did not receive the entire dose due to catheter displacement (n = 3: patients 12, 19, and 20), transfusion-like reaction (n = 1: patient 18), and elevated D-dimer values (>20.000 ng/mL; normal value < 500) (n = 1: patient 11). The overall rate of AEs and SAEs reporting was highest during hospitalization for HepaStem administration (duration period from the infusion start date to discharge from the hospital) and decreased thereafter in the ensuing periods. The rates of AEs and SAEs have been normalized by calculating the patient-month incidence rates for each period (Tables 2–5).

AEs assessed as possibly related to HepaStem or portal catheter placement were considered as adverse drug reactions (ADRs). All patients except one presented at least one nonserious ADR. During hospitalization for HepaStem administration and the following postinfusion days, mild nonserious ADRs included laboratory abnormalities and common features like nausea, vomiting, abdominal pain, or local pain. Anticoagulation administered during HepaStem infusion was well tolerated and no side effects related were observed. Clinical and vital signs including pulse oximetry as well as biological parameters were regularly monitored. Portal vein pressure following infusions remained within normal ranges.

Serious ADRs related to HepaStem administration or infusion catheter occurred in 7 patients during or after the infusion (Table 6). These SAEs included portal vein

T2-T5

T6



**FIGURE 1.** Dose escalation. The dose escalation was performed in both intra and intercohorts. Intracohort: The lowest dose was given to start the dose escalation. The administration of a dose had to be proven safe at least once before the administration of the next higher dose within the same weight cohort. Intercohort: The procedure and the treatment of one given dose had to be declared safe in a weight cohort before the same dose was administered to the next lower weight cohort.

**TABLE 2.**

**Incidence of adverse events by safety period and by disease**

Period	UCD N = 14	CN N = 6	Total N = 20
Before infusion			
Number of events	8	3	11
Number of patients with at least one event, n (%)	5 (35.7)	2 (33.3)	7 (35.0)
Reactogenicity [V infusion–V discharge <sup>a</sup> ]			
Number of events	68	33	101
Number of patients with at least one event, n (%)	14 (100.0)	6 (100.0)	20 (100.0)
Short-term safety [V discharge <sup>a</sup> –V D30]			
Number of events	44	9	53
Number of patients with at least one event, n (%)	14 (100.0)	4 (66.7)	18 (90.0)
Mid-term safety [V D30–V M6]			
Number of events	104	40	144
Number of patients with at least one event, n (%)	14 (100.0)	6 (100.0)	20 (100.0)
Prospective period [0–6 mo]			
Number of events	216	82	298
Number of patients with at least one event, n (%)	14 (100.0)	6 (100.0)	20 (100.0)
Long-term safety [V M6–study termination]			
Number of events	94	17	111
Number of patients with at least one event, n (%)	13 (92.9)	5 (83.3)	18 (90.0)
Prospective period [0–study termination]			
Number of events	310	99	409
Number of patients with at least one event, n (%)	14 (100.0)	6 (100.0)	20 (100.0)

<sup>a</sup>Actual discharge from hospital.

CN, Crigler-Najjar syndrome; D, day; M, month; UCD, urea cycle disorder; V, visit.

thrombosis (patients 3 and 10), transfusion reaction (patient 18), transaminase flare (patient 11), and metabolic decompensation (patients 3, 9, 11, 1, and 5). These 7 patients received a total number of HepaStem ranging from 0.765 to 4 billion cells infused over several days except for 1 patient.

Symptomatic metabolic decompensation occurred in 5 UCD patients (1 between infusions and 4 at days 2 to 4 postinfusion), involving 3 OTCD female adolescents (patients 1, 3, and 9), one 10-year-old male ASLD (patient 5), and one 7-year-old female ARGD (patient 11) who also exhibited transient transaminase increases. These events were resolved within a few days. None of the 5 patients with symptomatic decompensation required specific preventive intensified UCD treatment during infusion (ie, intravenous arginine and nitrogen scavengers,

transiently reduced-protein diet), whereas those who did not display metabolic decompensation had preventive intensification of the metabolic treatment. Of the 3 cited OTCD adolescent female patients with symptomatic metabolic decompensation, one developed left portal vein thrombosis (patient 3), after receiving the highest number of cells per day (3.5 billion over 2 days). No flow was detected in the left portal vein on the third infusion day, although pressure measured in the portal catheter remained normal. This patient was assigned to the intermediate dose and received the full dose. Another male adolescent OTCD patient (no 10), developed intraluminal nonocclusive parietal thrombus of the main portal vein after the removal of the transhepatic catheter that had remained in place for 5 days, the longest duration of catheter placement in this study. No previous abnormalities

**TABLE 3.****Incidence of serious adverse events by safety period and by disease**

Period	UCD (N = 14)	CN (N = 6)	Total (N = 20)
Before infusion			
Number of events	0	2	2
Number of patients with at least one event, n (%)	0 (0.0)	1 (16.7)	1 (5.0)
Reactogenicity [V infusion–V discharge <sup>a</sup> ]			
Number of events	5	2	7
Number of patients with at least one event, n (%)	4 (28.6)	1 (16.7)	5 (25.0)
Short-term safety [V discharge <sup>a</sup> –V D30]			
Number of events	6	0	6
Number of patients with at least one event, n (%)	5 (35.7)	0 (0.0)	5 (25.0)
Mid-term safety [V D30–V M6]			
Number of events	14	6	20
Number of patients with at least one event, n (%)	8 (57.1)	3 (50.0)	11 (55.0)
Prospective period [V infusion–6 mo]			
Number of events	25	8	33
Number of patients with at least one event, n (%)	10 (71.4)	4 (66.7)	14 (70.0)
Long-term safety [V M6–study termination]			
Number of events	19	1	20
Number of patients with at least one event, n (%)	6 (42.9)	1 (16.7)	7 (35.0)
Prospective period [V infusion–study termination]			
Number of events	44	9	53
Number of patients with at least one event, n (%)	11 (78.6)	4 (66.7)	15 (75.0)

<sup>a</sup>Actual discharge from hospital.

CN, Crigler-Najjar syndrome; D, day; M, month; UCD, urea cycle disorder; V, visit.

**TABLE 4.****Rate of AEs per patient-month by period**

Period	UCD	CN	Total
Reactogenicity [V infusion–V discharge*] (days)	n = 14	n = 6	n = 20
Number of AEs per patient-month mean ± SD	29.17 ± 16.91	46.56 ± 17.56	34.38 ± 18.54
Short-term safety [V discharge*–V D30] (days)	n = 14	n = 6	n = 20
Number of AEs per patient-month mean ± SD	3.58 ± 2.83	1.69 ± 1.36	3.01 ± 2.60
Mid-term safety [V D30–V M6] (days)	n = 14	n = 6	n = 20
Number of AEs per patient-month mean ± SD	1.45 ± 0.65	1.30 ± 0.32	1.40 ± 0.57
Prospective period [V Infusion–6 months] (mo)	n = 14	n = 6	n = 20
Number of AEs per patient-month mean ± SD	2.48 ± 0.99	2.22 ± 0.66	2.40 ± 0.89
Long-term safety [V M6–study termination] (mo)	n = 13	n = 5	n = 18
Number of AEs per patient-month mean ± SD	1.09 ± 0.66	0.53 ± 0.28	0.94 ± 0.63
Prospective period [V infusion–study termination] (mo)	n = 14	n = 6	n = 20
Number of AEs per patient-month mean ± SD	1.85 ± 0.79	1.56 ± 0.72	1.76 ± 0.76

AE, adverse event; M, month; UCD, urea cycle disorder; V, visit.

of the portal Doppler or portal pressures were observed. Both thrombi were treated using low-molecular weight heparin. The first thrombus was not resolved at month 12, with persistent left portal vein flow interruption, yet normal liver functional parameters. The second thrombus was fully resolved within 1 month. For these patients who developed different types of thrombosis in a portal vein, the anticoagulation was consistent with protocol guidelines, and activated clotting time results within expected margins during HepaStem infusions. The patients had a maximum increase in D-dimer values to 6200 (patient 3) and 11 500 ng/mL (patient 10).

One CN patient (patient 18) showed signs of a transfusion-like reaction. In the evening of the first infusion day, she developed fever with increased CRP and D-dimer values. The event was treated using corticoids (methylprednisolone) and anticoagulation overnight. A week later, infusions were restarted, and a similar event occurred, which resolved after stopping infusion.

Beyond this, 9 UCD and 3 CN patients presented SAEs not considered as ADRs (Supplementary data, SDC, <http://links.lww.com/TP/B674>).

In summary, a total of 298 AEs, including 33 SAEs, was reported for the 20 observed patients over a mean period

**TABLE 5.****Rate of SAEs per patient-month by period**

Period	UCD	CN	Total
Reactogenicity [V infusion–V discharge*] (days)	n = 14	n = 6	n = 20
Number of AEs per patient-month Mean ± SD	1.77 ± 3.57	0.92 ± 2.26	1.52 ± 3.20
Short-term safety [V discharge*–V D30] (days)	n = 14	n = 6	n = 20
Number of AEs per patient-month mean ± SD	0.48 ± 0.75	0.00 ± 0.00	0.34 ± 0.66
Mid-term safety [V D30–V M6] (days)	n = 14	n = 6	n = 20
Number of AEs per patient-month mean ± SD	0.18 ± 0.22	0.20 ± 0.31	0.19 ± 0.24
Prospective period [V infusion–6 mo] (mo)	n = 14	n = 6	n = 20
Number of AEs per patient-month mean ± SD	0.28 ± 0.25	0.22 ± 0.25	0.26 ± 0.25
Long-term safety [V M6–study termination] (mo)	n = 13	n = 5	n = 18
Number of AEs per patient-month mean ± SD	0.20 ± 0.25	0.03 ± 0.06	0.15 ± 0.22
Prospective period [V infusion–study termination] (mo)	n = 14	n = 6	n = 20
Number of AEs per patient-month mean ± SD	0.24 ± 0.20	0.13 ± 0.14	0.21 ± 0.19

AE, adverse event; D, day; M, month; UCD, urea cycle disorder; V, visit.

of 6.13 months. This led to a patient-month incidence rate of 2.4 for the AEs and 0.26 for the SAEs.

### Immunological Safety

Due to the allogenic origin of HepaStem, emergence of anti-HLA antibodies and their specificity to donor cells were evaluated at baseline, 6 and 12 months after infusion. Data showed that anti-HLA class I were present at baseline in 1 of 18 patients (patient 3) (Table 7). This anti-HLA was not specific to donor cells but belongs to the same cross-reactive group (CREG), this patient further developed donor-specific anti-HLA class I 6 months after cell infusion. The donor-specific anti-HLA class I were no more detected after 12 months. Anti-HLA class II antibodies were present at baseline in 1 of 18 patients (patient 17), not specific to donor cells and not detected 12 months after infusion. Four of 18 patients developed anti-HLA class I antibodies after infusion, from which 2 of 4 were donor-specific (patients 3 and 11) and 2 of 4 belong to the same CREG (patients 18 and 19). The donor-specific anti-HLA were detected 6 months after infusion and decreased at 12 months. High level of anti-HLA class I (mean fluorescence index > 5000) was observed in 1 of 18 patients (patient 11) 6 months after cell infusion and decreased at 12 months. The patients developing donor-specific anti-HLA were injected with 50 million cells/kg (patient 3) or 135 million cells/kg (patient 11), no donor-specific anti-HLA was observed in the patients injected with the lowest dose (12.5 million cells/kg). No patient developed donor-specific anti-HLA class II. The donor cells were found after 12 months in biopsies (FISH for Y chromosome—data not shown) from 2 patients developing either donor-specific anti-HLA class I antibodies or anti-HLA antibodies from the same CREG (patients 3 and 19, respectively).

### Preliminary Efficacy Evaluation

Patients exhibited variable baseline [<sup>13</sup>C] urea AUC-120 values, 9 exhibiting values between 4 and 51 μmol·min/L, clearly lower than healthy subjects (mean: 256 μmol·min/L, unpublished data), and 2 exhibiting 156 and 181 μmol·min/L, respectively (Figure 2, SDC, <http://links.lww.com/TP/B674>). One patient displayed a high baseline

value of 301 μmol·min/L, as yet unexplained. Given the high variability of baseline results and their normal distribution, an ANCOVA was performed with baseline [<sup>13</sup>C] urea AUC-120 as covariate (Figure 2 and Table 8). For the entire UCD group, this analysis revealed a statistically significant increase in the [<sup>13</sup>C] urea AUC-120 geometric mean at month 6 versus baseline (geometric mean ratio, 1.90; 95% CI, 1.22–2.97; *P* = 0.007). At month 3, all patients except 1 had positive ratios, whereas the change from baseline was statistically not significant (*P* = 0.409) as at month 12 (*P* = 0.214).

All CN patients were treated with prolonged (10 to 12 h) daily phototherapy. Baseline data revealed high variability, with individual values of 203.5 to 381.3 μmol/L (median: 312.95 μmol/L). All CN patients were referred to the principal investigator for the procedure, using other phototherapy devices while traveling out of their country (*n* = 5/6). Moreover, some patients' phototherapy devices were changed during the study as their initial devices were considered inappropriate (Figure 3A). For 2 patients (low dose, patients 16 and 19), ~20% decrease in total serum bilirubin levels could possibly be attributed to HepaStem when comparing periods using similar phototherapy devices (Figures 3A and B). It is interesting to mention that for patient 19 (female recipient of male cells, low dose), FISH performed on month 12 liver biopsy for gender chromosomes revealed 78% male cells (XY) and 12% recipient cells (XX) on 65 nuclei. For a third patient, the effect was transient (patient 18).

### DISCUSSION

This clinical trial study reports the safety primary endpoint and preliminary efficacy of HepaStem to treat rare IELM.

The UCD patients included had different enzymatic deficiencies and a variety of severe phenotypes. All showed low residual enzyme activity and markedly impaired protein tolerance. This population represented only a small fraction of the overall UCD population.<sup>18,19</sup> All were at high risk of sustaining metabolic decompensation even though they received therapy with nitrogen scavengers. Patients with CN syndrome displayed elevated serum bilirubin

**TABLE 6.****SAEs reported during the study**

System organ classification and preferred terms	Patients	Number of SAEs during study periods			Total
		Infusion period + few days	Up to 6 mo	6–12 mo	
Hepatobiliary disorders					
Portal vein thrombus	Patient 3 (UCD)	1	–	–	2
Left portal vein thrombosis	Patient 10 (UCD)	1	–	–	
Infections and infestations					
Gastroenteritis	Patient 1 (UCD)	–	–	1	6
	Patient 2 (UCD)	–	–	2	
	Patient 12 (UCD)	–	–	1	
	Patient 14 (UCD)	–	–	1	
	Patient 20 (CN)	–	1	–	
Laryngitis	Patient 16 (CN)	–	1	–	1
Infection (enteral)	Patient 14 (UCD)	–	–	1	1
Nasopharyngitis	Patient 13 (UCD)	–	1	–	1
Parainfluenza virus infection	Patient 13 (UCD)	–	1	–	1
Rhinovirus infection	Patient 13 (UCD)	–	–	1	1
Viral infection	Patient 14 (UCD)	–	–	1	1
Injury, poisoning, and procedural complications					
Transfusion reaction	Patient 18 (CN)	2	–	–	2
Gastrointestinal disorders					
Abdominal pain	Patient 3 (UCD)	–	–	1	1
Investigations					
Increased serum bilirubin	Patient 17 (CN)	–	4	–	4
Decreased coagulation factor	Patient 10 (UCD)	–	1	–	1
Decreased portal vein flow	Patient 3 (UCD)	1	–	–	1
Increased transaminases	Patient 11 (UCD)	1	–	–	1
Metabolism and nutrition disorders					
Hyperammonemia <sup>a</sup>	Patient 2 (UCD)	–	1	–	15
	Patient 3 (UCD)	1	1	3	
	Patient 8 (UCD)	–	1	–	
	Patient 9 (UCD)	1	4	–	
	Patient 11 (UCD)	1	–	–	
	Patient 12 (UCD)	–	2	–	
Metabolic disorder <sup>a</sup>	Patient 1 (UCD)	1	3	4	13
	Patient 5 (UCD)	1	–	–	
	Patient 12 (UCD)	–	–	1	
	Patient 13 (UCD)	–	1	1	
	Patient 14 (UCD)	–	–	1	
Benign, malignant, and unspecified neoplasms (including cysts and polyps)					
Mycosis fungoides	Patient 17 (UCD)	–	–	1	1
Total		11	22	20	53

<sup>a</sup>All considered as symptomatic metabolic decompensation.

CN, Crigler-Najjar syndrome; SAE, serious adverse event; UCD, urea cycle disorder.

levels, requiring constraining phototherapy impairing their quality of life.

This study sought to evaluate at different dosages (0.25% to 4.0% of total estimated liver cell mass<sup>20–22</sup>) the safety of both the product and the administration procedure. The safety profile corresponded to expectations for this new cell therapy,<sup>23</sup> infusion procedure, concomitant medications, age, and underlying diseases.<sup>24</sup> The data confirming the tolerability of HepaStem administered via the portal vein provided that precautionary measures were in place, especially for UCD patients.

During the course of HepaStem administration, all patients presented minor AEs, with a rate of about 1 AE

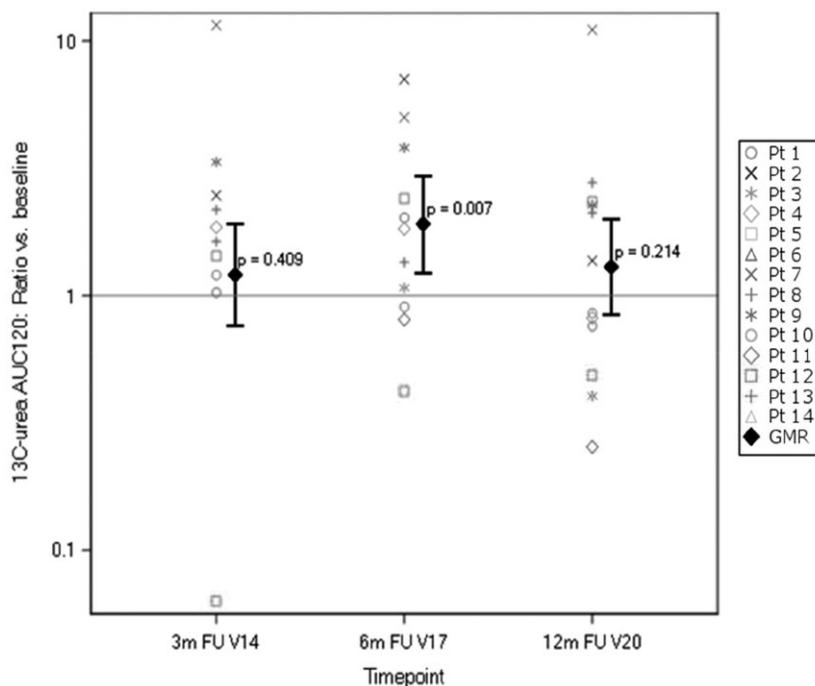
per day per patient. During the follow-up, the reporting rate decreased to about 1 or 2 AEs per month per patient. Infusion of a high number of HepaStem cells was associated with prolonged transhepatic intraportal catheterization, immobilization, fasting periods (in some cases), and hospitalization, likely increasing the risk of AEs, while lower doses given in one single day were well tolerated. Interestingly, younger patients displayed no increased risk of AEs. Indeed, these patients were treated with stringent supportive treatments, such as glucose and nitrogen scavengers given by intravenous route and/or reduced-protein diet.

Among 20 patients, 13 received the dose scheduled, 2 received the highest dose of  $200 \times 10^6$  cells/kg, while 3

**TABLE 7.****Anti-HLA antibody characterization**

Patient no	Anti-HLA baseline	Anti-HLA 6-mo visit	Anti-HLA 12-mo visit
Urea cycle defects			
<b>Patient 3</b>	<b>Anti-HLA class I: A1</b>	<b>Anti-HLA class I: A1, A3, A2, B56, B57, B67</b>	<b>Anti-HLA class I: A1</b>
<b>Patient 11</b>	Neg	<b>Anti-HLA class I: A2, A68, A69, A34, B7, B27, B54, B55, B56, B42, B81, B49, B50, B62, B57, B58, B67, B82, CW15</b>	<b>Anti-HLA class I: A2, B55, B56</b>
Crigler-Najjar			
<b>Patient 18</b>	Neg	<b>Anti-HLA class II: DR4</b>	<b>Anti-HLA class I: B7, B60, B81, B48</b> <b>Anti-HLA class II: DR4, DQA</b>
<b>Patient 17</b>	<b>Anti-HLA class II: DR10, DR4</b>	<b>Anti-HLA class II: DR4</b>	Neg
<b>Patient 19</b>	Neg	<b>Anti-HLA class I: AC-CL1N, A11</b>	<b>Anti-HLA class I: A11, B77</b>

AQ8 The text in bold indicates anti-HLA antibodies specific against donor cells.



**FIGURE 2.** Ureagenesis test.  $^{13}\text{C}$  urea AUC-120 ratio vs baseline in plasma at 3, 6, and 12 months are depicted. Month 6 vs baseline:  $P = 0.007$  (mixed-effect ANCOVA).

received the maximal dose of  $4 \times 10^9$  cells. Among the 7 patients who did not receive the allocated dose, 5 suffered from AEs or catheter displacement and infusions were stopped. These 5 patients were all allocated to the high dose, delivered over several infusions and several days. This emphasizes that infusions of low doses per day (ie, max  $500 \times 10^6$  cells), possibly repeated after days/weeks might be better tolerated and should constitute the basis of future trials.

Deep vein thrombosis prophylaxis remains a concern in all surgical or intravascular procedures. In liver transplantation, the incidence of portal vein thrombosis is estimated around 10%, and deep vein thromboses were also reported following bone marrow and hepatocyte transplantations.<sup>25-28</sup> Our data confirmed the risk of portal vein thrombus, which occurred in 1 UCD patient for whom the total infusion dose was given in a shorter time than

recommended. A second thrombus occurred postinfusion in a patient fitted with a curled catheter remaining in situ for 5 days. These events emphasize the need for appropriate perfusion equipment and careful monitoring of portal flow during infusion. In future trials, a mesenteric port and catheter might be placed for several weeks.<sup>29</sup>

HHALPCs express tissue factor and induce the coagulation cascade.<sup>11</sup> AEs related to the procoagulant effect of these cells as well as for mesenchymal stem cells (MSCs) were reported in other clinical trials.<sup>5,30-32</sup> In future trials, cell doses should be carefully calculated, favoring low doses per day. Anticoagulation may remain necessary for intraportal infusion. With careful monitoring of the patient's parameters, one should not expect a thrombosis rate more elevated than with hepatocyte transplantation.

Metabolic decompensation was observed during HepaStem infusion in some UCD patients, yet not in

**TABLE 8.****AQ9 Ureagenesis test at baseline, 3, 6, and 12 months**

Disease/patient no	Dose	Cohort	Baseline	3 months		6 Months		12 Months	
			AUC	AUC	Ratio (3 mo/BL)	AUC	Ratio (6 mo/BL)	AUC	Ratio (12 mo/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).

children prophylactically placed under protective metabolic support, as recommended during intercurrent illnesses or medicosurgical procedures. In this trial, a bolus of methylprednisolone was used as an initial immunosuppression. Although corticosteroids are known to induce endogenous protein catabolism when chronically administered, single doses can be safely administered to UCD patients.

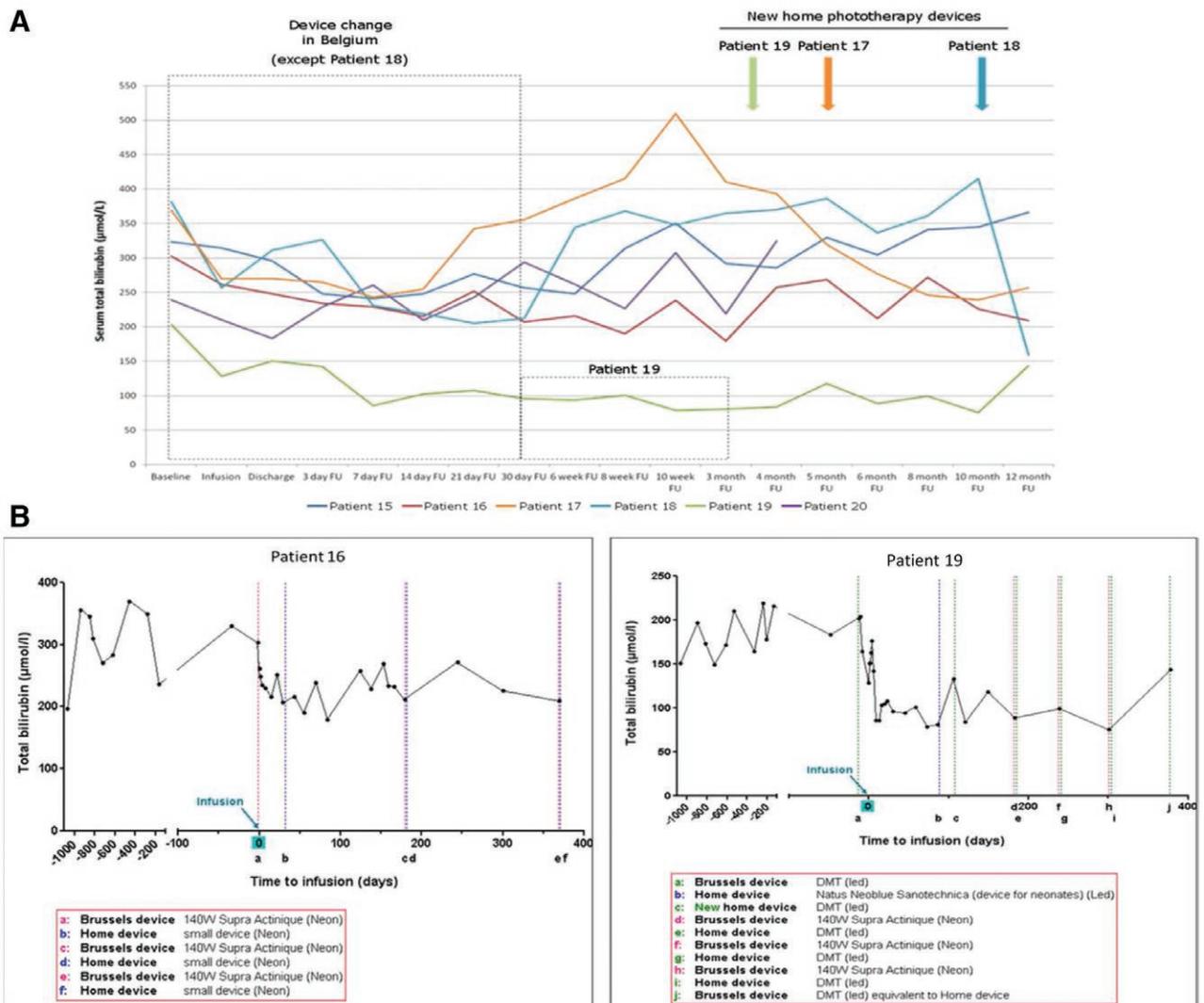
In the allogeneic scenario, the expression of relevant immune molecules, notably the polymorphic major histocompatibility complex class I and II molecules (HLA class I and II in humans), is recognized to induce rejection. An important component of allograft failure in organ transplantation is antibody-mediated rejection.<sup>33,34</sup> It results from the interaction of antibodies against mismatched donor antigens with the allograft vascular endothelium. Allosensitization to non-self highly polymorphic HLA is a major limitation of effective clinical organ, tissue, and cell transplantation. Liver-derived MSCs exhibit low immunogenicity and exert in vitro a similar immunoregulatory effect as bone marrow-derived MSCs.<sup>35,36</sup> Indeed, liver-derived MSCs expressed a low level of HLA class I and no HLA class II even in a pro-inflammatory environment.<sup>37,38</sup> Donor-specific anti-HLA class I antibodies were detected only in 2 patients, whereas no donor-specific anti-HLA class II antibodies were detected. Anti-HLA class I from the same CREG as the donor cells was detected after cell infusion; however, their significance remains uncertain. The prevalence of de novo DSA was much lower compared with what has been described for pediatric patients after ABO-compatible liver transplant and clinical trials using allogeneic adipose tissue-derived MSCs.<sup>39,40</sup> Moreover, donor cells were detected in patients in which donor-specific anti-HLA class I

antibodies were induced, indicating that the presence of donor-specific anti-HLA antibodies do not impair donor cell engraftment.

UCD diseases are influenced by compliance with treatment and by intercurrent stress such as acute infection. Therefore, detecting HepaStem efficacy might be difficult. The in vivo functional <sup>13</sup>C ureagenesis test investigating urea cycle functionality is expected to be an independent measurement without interference from other parameters like treatment compliance.<sup>41</sup> Opladen et al showed that the test allowed delineation of different degrees of urea cycle deficiency in neonatal onset patients versus late onset patients and carriers.<sup>42</sup> This test was previously used to assess treatment efficacy.<sup>43,44</sup> In this study, increased ureagenesis was observed in most UCD patients following HepaStem infusion, with overall statistically significant improvement of the de novo urea formation at the 6-month postinfusion evaluation.

This study provided also important insights for designing future efficacy study in UCD and CN patients. The quality of the available medical history was variable. Prospective data showed that UCDs and CN are complex diseases evolving with fluctuations in which intercurrent events and patient-specific supportive treatments had high impacts. Adolescence is a known at-risk period for all chronic diseases, due to growth, hormonal factors, necessary metabolic adjustments, and decreased compliance.<sup>45</sup> Therefore, to distinguish HepaStem effects in these diseases, future trials should include prospective baseline and follow-up periods including similar monitoring.

In conclusion, this study confirms HepaStem's tolerability in pediatric patients displaying IELM. It provided that the patients can prophylactically receive individualized



**FIGURE 3.** Bilirubin levels at scheduled visits for CN patients. (A) Serum total bilirubin profiles at follow-up ( $\mu\text{mol/L}$ ). 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 19. Dotted lines: changes in phototherapy devices.

metabolic support with careful monitoring of clinical and biochemical status. This paves the way for future investigations to explore HepaStem efficacy in UCD, CN, or new indications. Cell therapy has so far shown a favorable safety profile, but safety assessment description has, in general, been of poor quality, and only AEs that are looked for will be found.<sup>46</sup> To our knowledge, this is the first time that a safety assessment description has been reported in detail up to a 12-month follow-up.

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AQ1—Please check and approve the edits made to the article title.

AQ2—For indexing purposes, please confirm that author names have been correctly identified as given names (blue) and surnames (red). Color in the byline will not appear on the final published version.

AQ3—Please check and approve the edits made to the sentence "HepaStem was granted with the advanced therapy medicinal ...".

AQ4— Please check and approve the edits made to Table 1A.

AQ5— Please check and approve the edits made to Table 1B and its footnote.

AQ6—Please provide expansion for "CRD".

AQ7— Please provide significance for "\*" used in Table 5.

AQ8—Please check and confirm the changes made to footnote of Table 7.

AQ9—Please provide expansion for "DSA".

AQ10—Please check and approve the edits made to Table 8.