SUPPLEMENTARY MATERIALS AND METHODS

Detailed assays for each indication are here below listed:

UCD:

- Metabolic status: Ammonium levels and plasma amino acids (alanine, arginine, citrulline, glutamine; glutamate, glycine)
- Orotic acid (OTCD only) or argininosuccinic acid (ASLD only) in urine.
- Metabolic decompensation defined as hyperammonaemia associated with clinical symptoms.
- Supportive treatment and any adjustment of diet (protein restriction [low protein diet)] and amino acids supplements),
- ^{13}C tracer method to measure ureagenesis at baseline, 3, 6, and 12 months post infusion for selected patients. Briefly, this method used stable non-radioactive isotopes to evaluate actual urea cycle activity, incorporating ^{13}C into urea from ^{13}C-labeled precursor[18-20]. UCD patients ingested 0.33 mmol/Kg (27 mg/Kg) sodium [1-^{13}C] acetate dissolved in 60 mL 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). The samples were evaluated at the central laboratory (Children's Hospital of Philadelphia, USA).

CN:

- Supportive treatment and any adjustment of duration of phototherapy,
- Serum unconjugated (indirect) bilirubin.

SUPPLEMENTARY RESULTS

Nature of the events

Nine UCD and 3 CN patients presented SAEs not considered as ADRs. They included episodes requiring hospitalization for common infections and/or underlying disease decompensation, in line with age and morbidity of the studied population. Reported infections were primarily upper respiratory tract and gastrointestinal infections. In total eight UCD patients displayed symptomatic metabolic decompensations treated at hospital during this period. Five of them (Patients 2, 8, 12, 13 and 14) displayed 1-2 episodes. Additionally, three female OTCD adolescents (patients 1, 3 and 9) suffered between 5 and 7 episodes, with non-compliance to supportive treatment reported in one patient (patient 1). Among CN patients, one CN1 (patient 17) 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 (Patients 17 and 19) developed skin depigmentation areas on the limbs after month 12, one diagnosed with mycosis fungoides (patient 17) based on histology, yet without molecular rearrangement or genetic abnormalities revealed on skin biopsy. As the combination of tacrolimus and phototherapy was the suspected cause, tacrolimus was stopped and both events resolved in both patients. All SAEs were treated adequately and resolved except one case of left portal vein thrombosis. Young patients were not at increased risk of SAEs.

Percutaneous liver biopsies were performed at baseline, months 6 and 12. No complications were observed during or following biopsies. At baseline, about a quarter of patients presented fibrosis with a Metavir score[17] of 1, 1 patient had a score of 3 and the other patients had score of 0 (Supplementary Table 1). At 6 and 12-month FU, no increased fibrosis scores were observed. Patient 12 had a fibrosis score of 1/0 (baseline), 2/1 (at 6-month) and 0/0 (at 12-month). Inflammation scores varied between 0 and 1 at each time point. No safety signal related to either liver fibrosis or inflammation was observed for any of the patients infused with HepaStem®.

Glutamine assessments revealed intra- and inter-patient variability with values stabilizing over time and varying between 800 (upper normal range for healthy individuals) and 1000 μmol/L (clinically-acceptable threshold for UCD patients). Median glutamine value at baseline was 915 μmol/L (range: 361-1496 μmol/L), and at last follow-up 1082 μmol/L (range: 267-1330 μmol/L).

Preliminary efficacy evaluation

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

Individual ammonia profiles revealed inter-patient variability, fluctuating primarily below 80 μmol/L with random peaks up to 411 μmol/L (Supplementary Figure 1). The median ammonia value at baseline was 49 μmol/L (range: 19-144 μmol/L), and at last follow-up 53 μmol/L (range: 5-125 μmol/L). All patients received at least one ammonium scavenger, and half received both sodium benzoate and sodium phenylbutyrate. Overall, the drug doses/Kg, were stable. In those treated with ammonium scavengers for 3 months pre-cell-infusion, the median dose of sodium benzoate was 212 mg/Kg/day (range: 104-449 mg/Kg/day), and that of sodium phenylbutyrate 275 mg/Kg/day (range: 99-543 mg/Kg/day). Over the last three months, the median sodium benzoate dose was 208 mg/Kg/day (range: 115-419 mg/Kg/day), and that of sodium phenylbutyrate 231 mg/Kg/day (range: 81-419 mg/Kg/day).

Supplementary Table 1: METAVIR scores per patient at baseline, 6 month and 12 month visits

Patient ID	Inflammation: A score			Fibrosis: F score		
	Baseline	6-month visit	12-month visit	Baseline	6-month visit	12-month visit
Urea Cycle Defects						
CPSID						
12	0/0	0/0	0/0	1/0	2/1	0/0
13	0/0	0/0	0/0	0/0	0/0	0/0
OTCD						
1	0/0	0/0	1/0	0/0	0/0	0/0
2	0/0	0/0	0/0	0/0	0/0	0/0
3	0/0	1/0	0/0	1/1	1/0	1/1
4	0/0	0/0	0/0	0/0	0/1	0/1
6	0/1	DO	DO	0/0	DO	DO
8	0/1	0/1	0/0	0/1	0/0	0/1
9	0/1	ND	0/0	0/0	ND	0/0
10	0/1	ND	ND	0/1	ND	ND
14	ND	MD/1	MD/1	ND	3/2-3	3/3
ASLD						
5	0/0	0/1	1/0	3/1	3/0	(0)*/0
7	1/1	1/1	MD/1	3/3	3/1	3/3
ARGD						
11	0/0	1/0	0/0	0/0	0/0	0/0
Crigler Najjar						
Type I						
15	0/0	1/0	1/MD	1/0	1/0	0/MD
16	0/0	0/0	0/1	2/0	0/0	0/0
17	0/0	0/0	1/0	0/0	0/1	1/0
18	0/0	1/MD	1/0	0/0	2/MD	1/1
20	0/1	R	DO	1/1	R	DO
Type II						
19	0/0	0/0	0/0	0/0	0/1	1/1

Score x/y: Score provided by local pathologist/score provided by central pathologist

*At 12 months: suboptimal tissue - high risk of underscoring

DO, liver biopsy not done because patient dropped out; MD, liver biopsy done but score not provided; ND, liver biopsy not done

SUPPLEMENTARY FIGURE 1