

CLINICAL AND BIOCHEMICAL PARAMETERS

Overall, the graphical representation taking into account the retrospective data collected at screening showed repeated variations (increased/decreased) in daily protein intakes for the 3 patients included in the SS. Only one patient received a total dose of protein which was in line with the 'WHO safe level' for protein intake.

Protein intake and supplementation

During the baseline period (N=3), the protein intake appeared to be stable (median natural and total protein being respectively 600 mg/kg/day and 820 mg/kg/day. As of FU visit 3 (6 months post first infusion), the investigators were requested to increase the daily total protein dose adjusted to BW. Evolution in protein intake during the study participation was as follows:

- Patient with ASLD: Daily total protein doses were first decreased during PAC placement, treatment and recovery period following the PAC removal (from 820 mg/kg/day at the last baseline visit to 692 mg/kg/day at the 3-month FU visit). Then, they were increased from 6 months post infusion 1 till the 9-month FU visit (from 580 to 645 mg/kg/day). The patients received essential amino acid supplementation throughout the study periods. Daily nitrogen scavenger and arginine doses remained stable from baseline till end of the study.
- Patient with CPSID: the daily dose of total protein was progressively increased during the whole FU period (from 470 mg/kg/day to 800 mg/kg/day at the 10.5-month FU visit. However, an emergency diet was to be started at 12 months post first infusion. The patient received essential amino acid as well as citrulline and arginine supplementation. The daily doses of nitrogen scavenger and citrulline were maintained till study discontinuation but the daily dose of arginine was reported as decreased after 2.5 months of FU. Of note, a non-serious metabolic decompensation (with increased ammonia value) was reported for this patient at the end of the FU period.

The protein intake for these 2 patients remained largely below the WHO safe level of protein intake for children during the follow-up period (between 580 and 690 mg/kg/day versus 900 mg/kg/day).

Table: Protein intake over the baseline and follow-up periods – Safety set (N=3)

	Daily dose (mg/kg/day)	Total Protein	Natural protein
Baseline period			
Visit 1		N=3	N=3
	Mean (SD)	758.9 (263.8)	478.5 (430.8)
	Median [range]	820.0 [470 ; 987]	600.0 [0 ; 836]
Visit 2		N=3	N=3
	Mean (SD)	762.1 (297.4)	478.5 (430.8)
	Median [range]	820.0 [440 ; 1026]	600.0 [0 ; 836]
Visit 3		N=3	N=3
	Mean (SD)	752.8 (141.7)	422.2 (367.2)
	Median [range]	820.0 [590 ; 848]	600.0 [0 ; 667]
Follow-up period			
FU 1 (3 months)		N=2	N=2
	Mean (SD)	616.2 (107.8)	273.1 (386.2)
	Median [range]	616.2 [540 ; 692]	273.1 [0 ; 546]
FU 3 (6 months)		N=2	N=2
	Mean (SD)	581.4 (2.0)	223.5 (316.1)
	Median [range]	581.4 [580 ; 583]	223.5 [0 ; 447]
FU 5 (9 months)		N=2	N=2
	Mean (SD)	667.3 (32.1)	294.7 (303.7)
	Median [range]	667.3 [645 ; 690]	294.7 [80 ; 509]
FU 9 (12 months)		N=1	N=1
	Mean (SD)	630.0 (NA)	0.0 (NA)
	Median [range]	NA	NA

FU: follow-up, SD: standard deviation; NA: not applicable

Blood tests

Ammonia is the most frequently used biochemical parameter in UCD patients for diagnosis and monitoring of the clinical metabolic condition. Plasma amino acids are also contributing diagnosis and monitoring tools. In non-treated (or non-compliant or unstable) UCD patients, amino acids in the metabolic pathway immediately upstream to the enzyme defect increase, and those beyond the block decrease in blood.

Blood parameters were evaluated in the SS i.e. for patients with ASLD, ASSD and CPSID. In those patients, glutamine and alanine accumulate while arginine is decreased. Citrulline is decreased in CPSID and increased in ASLD and ASSD.

Retrospective ammonia and amino acid data were collected for 3 years before HepaStem infusion. This included mainly field measurements performed at irregular time points. During the study, ammonia and amino acid levels were measured at each BL and FU visit. In addition, ammonia was measured at each visit during the active treatment period while an amino acidogram was performed at the beginning of the stabilisation periods following the PAC placement and removal.

During the study periods, if several samples were collected on the same day, the median value by fasting status was considered for the analysis.

All patients received essential amino acid supplementation throughout their study participation.

Ammonia

According to the UCD guidelines published by European experts (Häberle 2012), plasma ammonia should remain < 80 µmol/L in UCD patients. When ammonia values are above 100 µmol/L, it is recommended to stop protein intake and to initiate IV medication.

Based on the retrospective ammonia values, individual patient median, percentile 10 (P10) and percentile 90 (P90) were calculated.

Retrospective data showed that ammonia levels were usually maintained below the recommended 80 µmol/L (individual period median ranging between 34.6 and 65.0 µmol/L). A high variability was observed on the upper values (maximum period ammonia ranging between 78.0 and 575.0 µmol/L) reflecting the occurrence of episodes of hyperammonaemia in some patients (in 2 out of 3 patients).

Accordingly, inter-patient variability was observed during the study period (prospective collection of data).

- The patient with ASSD maintained ammonia blood levels below 50 µmol/L throughout his study participation (BL period and first HepaStem infusion).
- The patient with ASLD presented a high ammonia level at baseline which decreased throughout the study periods although oscillating between 19 and 86 µmol/L. During the FU period, ammonia levels appeared to be maintained below 80 µmol/L.
- The patient with CPSI presented ammonia levels below 80 µmol/L during the baseline period but experienced several episodes of hyperammonaemia during the active treatment period and at the end of the FU period. One episode of hyperammonaemia and one episode of metabolic decompensation clinically asymptomatic were reported as AEs for this patient during the active treatment and follow-up periods.

Table: Ammonia levels (µmol/L) before screening (retrospective data collection) - Safety set (N=3)

	Median	P10	P90	Min	Max
N	3	3	3	3	3
Median	48.000	27.0000	153.0000	14.0000	427.0000
range	34.600; 65.000	12.500 ; 31.000	78.000 ; 162.000	12.500 ; 20.000	78.000 ; 575.000

P10: Percentile 10; P90: Percentile 90

Glutamine

In normal subjects, normal range of plasma glutamine is 480-800 µmol/L and upper normal range of plasma glutamine is around 800 µmol/L (676 to 879 µmol/L, depending on patient's age and laboratory in the study centres). According to the UCD guidelines published by European experts (Häberle 2012), plasma glutamine should remain < 1000 µmol/L in UCD patients.

Based on the retrospective glutamine blood levels, individual patient median, P10 and P90 were calculated.

Retrospective data showed a high variability between patients with individual medians varying from 465.0 to 1202.0 µmol/L (median: 755.0 µmol/L), the highest levels being observed for the patient with CPSID.

High variability was also observed during the study periods (after screening). The patients with ASLD and ASSD maintained relatively stable glutamine blood levels which remained below 1000 µmol/L. The third patient (with CPSID) had uncontrolled glutamine levels which ranged between 250 and 2000 µmol/L.

Table: Glutamine levels (µmol/L) before screening (retrospective data collection) - Safety set (N=3)

	Median	P10	P90	Min	Max
N	3	3	3	3	3
Median	755.00	410.00	980.00	307.00	1125.0000
range	465.0 ; 1202.0	320.0 ; 875.0	647.0 ; 2418.0	215.0 ; 875.0	693.0 ; 2418.0

P10: Percentile 10; P90: Percentile 90

Alanine

Alanine serves as a transporter of ammonia and may accumulate in non-controlled UCD patients. Depending on laboratory and age groups, lower limit of normal ranges varies between 120-258 µmol/L and higher limit varies between 400-600 µmol/L.

Based on the retrospective alanine blood values, individual patient median, P10 and P90 were calculated.

Overall, individual levels collected retrospectively remained within or just above the defined higher limit (< 700 µmol/L).

Individual profiles during the study periods exhibited a similar pattern to the one observed for glutamine: The patients with ASLD and ASSD maintained relatively stable levels which remained below 400 µmol/L. Values recorded for the patient with CPSID appeared to be uncontrolled with large fluctuations and levels ranging between 250 and 800 µmol/L.

Table: Alanine levels (µmol/L) before screening (retrospective data collection) - Safety set (N=3)

	Median	P10	P90	Min	Max
N	3	3	3	3	3
Median	367.00	256.00	510.00	230.00	580.00
range	310.0 ; 449.8	145.0 ; 308.0	458.0 ; 691.0	80.0 ; 308.0	468.0 ; 691.0

P10: Percentile 10; P90: Percentile 90

Citrulline

Depending on laboratory and age groups, lower limit of normal ranges is 10 µmol/L and higher limit varies between 200 and 300 µmol/L.

Based on the retrospective citrulline blood level, individual patient median, P10 and P90 were calculated.

As expected, retrospective data showed low values of blood citrulline before study inclusion for the patient with CPSID (individual median value: 33,86 µmol/L) while the patients with ASLD had value closed to the upper normal range value (individual median value: 170 µmol/L) and patients with ASSD exhibited very high values (individual median value: 1205 µmol/L).

Citrulline values recorded during the study periods were in line with those reported retrospectively: Values remained above the upper normal range value during the treatment period for the patient with ASLD and tended to further increase at the end of the FU period. Citrulline blood values remained above the upper normal range value for the ASSD patient. Citrulline concentrations remained low for the patient with CPSID, however, levels tended to increase a couple of weeks before treatment start. This last patient received supplementation of citrulline, arginine and essential amino acid throughout the study periods. No dose adjustments were reported for citrulline during the FU period. A dose adjustment was reported in arginine 73 days after first HepaStem infusion.

Arginine

Depending on laboratory and age groups, lower normal ranges varied between 10-64 $\mu\text{mol/L}$ and higher normal ranges varied between 125-160 $\mu\text{mol/L}$.

Based on the retrospective arginine blood levels, individual patient median, P10 and P90 were calculated.

Retrospective data showed that individual median for the period preceding study inclusion remained below the upper limit of the normal range. However, a median maximum value largely above 160 $\mu\text{mol/L}$ was reported for the patient with ASLD (median maximum: 460 $\mu\text{mol/L}$).

During the study, arginine values remained within normal ranges for the patients with ASSD and ASLD during their participation. The patient with CPSID presented values within normal range at BL which sharply increased during the BL and active treatment periods and seemed to be oscillating during the FU period (between 75 and 200 $\mu\text{mol/L}$). Dose adjustment (decrease) in arginine was reported approximately 2.5 months after first infusion of HepaStem.

Of note, the 3 patients took arginine supplementation during their study participation.

Table: Arginine levels ($\mu\text{mol/L}$) before screening (retrospective data collection) - Safety set (N=3)

	Median	P10	P90	Min	Max
N	3	3	3	3	3
Median	58.00	30.00	197.00	20.00	201.00
range	53.5; 159.0	25.0 ; 45.5	138.2 ; 270.0	9.0 ; 30.0	138.20 ; 460.0

P10: Percentile 10; P90: Percentile 90

Growth indicators

Retrospective data were collected in order to have a more global picture of the patients' growth. Overall (i.e. collected retrospectively and during the study), height remained between the 50th and the 5th percentiles CDC reference curve. Two (2) patients (ASLD and ASSD patients) experienced a slight dropout. The decrease was smooth and occurred over a period of at least one year. The patient with ASSD seemed to have caught up some height gain over the study period. The dropout in height was associated to a dropout in weight for the patient with ASLD. However, weight remained above the 25th percentiles CDC curves.

Other height, weight and head circumference profiles remained within normal range of CDC reference curves with some moderate fluctuations.

Cognitive skills

Evolution in the patients who complete the active treatment and follow-up period was as follows:

- The patient with ASLD was rated as severely ill at screening. Wechsler score reflected mental impairment both at BL and at the 12-month FU visit (Wechsler score at baseline: 44; category mentally impaired). At the 3-month FU visit and up to the end of the FU period, the investigator judged that the status of the patient has improved to markedly ill. However, at the end of the FU period a neurological decompensation was reported.

The investigator's clinical global impression was minimal improvement with an efficacy score of 11. This score was increased to 15 at the 12-month FU visit.

- No evidence of mental impairment was reported for the patient with CPSID at baseline or at any of study visits (Wechsler score at baseline: 94; category: average).

Following HepaStem infusion, the investigator's clinical global impression was a really improved condition with an efficacy score of 7.