1 SYNOPSIS

Name of sponsor/company: Cytonet GmbH & Co. KG	**Full clinical trial report in dossier section:**	**(For National Authority Use only)**
Name of finished product: HHLivC (for infusion)		
Name of active ingredient: Human Heterologous Liver Cells (for infusion)		

Number of trial and title: CCD02
Open, Prospective, Uncontrolled, Multicentre Study to Evaluate the Safety and Efficacy of Multiple Applications of Liver Cell Suspension Into The Portal Vein in Children with Urea Cycle Disorders (UCDs)
Protocol ID:
The study was based on protocol version 5.0 (12 June 2013) and its amendment dated 21 September 2015

EudraCT number:
2006-000136-27

Coordinating investigator:
Prof. Dr. med. Prof. h.c. mult. (RCH) Georg Friedrich Hoffmann

Trial centres:
The study was conducted in 2 active centers in Germany:
- Univ.-Prof. Dr. med. Prof. h.c. mult. (RCH) Georg F. Hoffmann, Universitätsklinikum Heidelberg
- Dr. Andrea Schlune, Universitätsklinikum Düsseldorf

Publication (reference):
Not applicable.

Phase of development:
II

Trial period:
From August 21, 2009 (first patient in study) to December 30, 2013 (last patient final visit)

Trial objectives:
Objective was to investigate the safety and efficacy of multiple applications of liver cell suspension in children with urea cycle disorders.
The primary variables were:
- Safety of the application of liver cells as measured by oxygen saturation, portal blood pressure and flow during the infusion
- Safety of the placement of an application catheter to the portal vein

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- Safety of catheter insertion as determined by the evaluation of all adverse events after liver cell infusion (protocol version 5.0, before amendment dated 21 September 2015)
- Safety of the placement of an application catheter to the portal vein by evaluation of all adverse events judged to be related to the catheter placement (as per amendment dated 21 September 2015)

Methodology (Design of the study)
Open, prospective, uncontrolled, multicentre phase II study with multiple applications of liver cell solutions.

Number of patients
Planned: 12 evaluable patients (no more than 20 included)
Analysed: 12 patients

Diagnosis and main criteria for inclusion and exclusion:
Patients might be included into the study if one of the following bullet points were fulfilled :

- Neonates and infants up to the age of ≤3 months with prenatally or postnatally confirmed urea cycle disorder or
- Children aged >3 months up to ≤5 years of age with unstable metabolism and confirmed urea cycle disorder of either:
  - Carbamylphosphate synthetase I (CPS1D) or
  - Ornithine transcarbamylase (OTCD) or
  - Argininosuccinate synthetase deficiency (ASSD/Citrullinaemia)

A DNA analysis further confirmed diagnosis prior to or after inclusion according to the protocol.
Additional criteria required:

- Accessibility of the portal vein
- Plasma ammonia level ≤250 µmol/l
- Written informed consent from parents or legal guardians

A patient might not be included into the study if any one of the following criteria was fulfilled:

- Structural liver disease (cirrhosis, portal hypertension), or venoocclusive diseases
- Portal vein thrombosis
- Body Weight ≤3.5 kg
- Carrier of the human immunodeficiency virus (HIV)
- Any other contraindication for immunosuppression
- Presence of acute infection at the time of inclusion

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- Participation in other clinical trials or received experimental medication within the last 30 days
- Live vaccination planned during the course of the study
- Live vaccination within 4 weeks prior to beginning of study
- Allergic disposition against contrast medium used in study and/or antibiotics used in the manufacturing process
- Required valproate therapy
- Severe coagulopathy or thrombocytopenia
- Known diagnosis of hereditary thrombophilia (e.g. Factor V Leiden, Prothrombin 20210A variant) or parental history of hereditary thrombophilia and absence of thrombophilia testing in subject
- Cancer, severe systemic or chronic disease other than study indication (urea cycle deficiency)

Test product(s), dose and mode of administration, batch numbers:
Human Heterologous Liver Cells (HHLivC) for infusion, application into the portal vein via a Hickman/Broviac catheter introduced into branches of the inferior or superior mesenteric vein by surgery.
Cell dosage (divided into 6 applications) for children who weigh:
≤10 kg: 0.3 x 10^9 viable liver cells per kilogram of body weight
>10 to 15 kg: 3.0 x 10^9 viable cells nonadjusted to body weight
>15 kg: 0.2 x 10^9 viable liver cells per kilogram of body weight

HHLivC batch no:
071006VLK004 (02, 03, 04, 05, 06)
071006VLK005 (02)
090222VLK004 (01, 02, 03, 04, 05, 06, 07, 08, 09)
071006VLK005 (03, 04, 06)
080806VLK033 (01, 02)
091109VLK016 (01, 02, 03, 04)
090222VLK004 (10)
080404VLK016 (01, 02, 03, 04, 05, 06, 07, 08, 09 10)
080404VLK017 (01, 03, 04, 05, 06, 07, 09, 10)
100127VLK003 (03, 14, 01, 07, 06)
100127VLK004 (01, 03, 05, 06, 07, 09,11, 02)
110130VLK004 (01, 02, 04, 05, 06, 07, 08, 09, 10, 13, 15)

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110404VLK019 (02) 110529VLK030 (03,06,07,08,12,14)		

Duration of treatment:
6 individual sessions per patient were intended to be performed. Duration of the HHLivC application phase was 6 days. The planned time span between sessions was approximately 24 hours.

Criteria for evaluation
Endpoints:
Primary safety variables:

- Safety of the application of liver cells as measured by oxygen saturation, portal blood pressure and flow during the infusion
- Safety of the placement of an application catheter to the portal vein
- Safety of catheter insertion as determined by the evaluation of all adverse events after liver cell infusion (before amendment dated 21 September 2015)
- Safety of the placement of an application catheter to the portal vein by evaluation of all adverse events judged to be related to the catheter placement (as per amendment dated 21 September 2015)

Secondary safety variables were:

- Vital signs
- Laboratory Parameters III to V (haematology, biochemistry, urinalysis, immunoglobulins, serology) to monitor the safety of the procedures and immunosuppression, and
- Adverse Events

Secondary efficacy variables were:

- Changes in ^{13}C urea formation from baseline compared to 2 and 4 months (or earlier, if OLT is performed during listing period) after first liver cell infusion and, if available, up to 24 months (FUV 5) after the Final Visit, in case further ^{13}C-ureagenesis tests were performed after infusion of HHLivC (per amendment to protocol version 3.0),
- Change in the respective enzyme activity in samples from the explanted liver taken after OLT compared to the enzyme activity in the liver biopsy taken prior to the first liver cell application,
- Detection of donor cell material in samples from the explanted liver taken after OLT

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compared with the liver biopsy taken prior to first liver cell application,

- Number, duration and severity of metabolic crises (maximal ammonia concentration, duration of coma),
- Laboratory parameters I and II: ammonia and amino acids in plasma and orotic acid in urine (except in CPS1D),
- Growth and protein intake
- Nutritional status
- Use of ammonia scavenging drugs and
- Time to death and survival at 6 month after liver cell infusion (per amendment to protocol version 5.0)

Exploratory variables were:

- Total urea and orotic acid (except CPS1D;) in 12-hour urine, and
- Urea in serum

Statistical methods:

Two analysis sets were used for the Interim Analysis I (after 5 patients) and Interim Analysis II (after 11 patients):

- The safety set (SAF) for all safety analysis, comprising all patients who had at least one attempt for placing an application catheter for liver cell suspension.
- The full analysis Set (FAS) for secondary efficacy endpoint analysis, including all patients of the safety set for whom any efficacy assessment(s) was/were available.

In the final analysis 5 sensitivity analysis subsets (Sens1 – Sens5) were added for the evaluation of secondary efficacy endpoints:

- The sensitivity analysis set 1 (Sens1) included all patients of the FAS, but excluded patients in whom LCT was not completed.
- The sensitivity analysis set 2 (Sens2) included all patients of Sens1, but excluded patients without neonatal onset of disease.
- The sensitivity analysis set 3 (Sens3) included all patients of Sens2, but excluded patients with a start of LCT prior to stabilization in the initial hyperammonaemic event.
- The sensitivity analysis set 4 (Sens4) included all patients of Sens2, but excluded patients with clinically significant treatment errors.
- The sensitivity analysis set 5 (Sens5) included all patients of Sens2, but excluded patients with a start of LCT prior to stabilization after initial hyperammonaemic event and patients with clinically significant treatment errors.

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Safety variables:
The safety of the application of liver cells as measured by oxygen saturation, portal blood pressure and flow during the applications was to be analysed for each infusion and patient. Descriptive statistics of absolute values for these continuous variables were to be computed for the start of application and for all available values until the end of application. Additionally, the difference of the last value to baseline was to be calculated and analysed descriptively.
The safety of the placement of an application catheter to the portal vein was to be displayed by the number and percentage of patients with successful and unsuccessful catheter placement, respectively. Furthermore, the safety of catheter insertion was to be displayed by evaluation of all adverse events judged to be related to the catheter placement (imputability assessment).
Adverse events of patients not receiving any liver cells and adverse events with onset before first attempt of catheter placement were to be included in the analyses. In addition these adverse events were to be listed separately. The incidence of serious adverse events and adverse events leading to withdrawal from study and/or treatment were also to be listed. Percentages were to be calculated relative to the number of included patients. The incidence and reasons for less than six sessions were to be given. Information about the percentage of prematurely terminated sessions and number of interruptions per sessions was to be summarised by descriptive statistics. The assessment of relatedness to study medication by the investigator was extended to sponsor assessment of the AE relationship to catheter placement, liver cell administration, and immunosuppressive treatment.

For vital signs, descriptive statistics were to be given for absolute values and differences to baseline by visit and the last available value prior to first OLT. Additionally, vital signs were to be summarised for each HHLivC application before and after liver cell infusion.
For laboratory III to V, descriptive statistics were to be given for absolute values and differences to baseline by visit and the last available value prior to first OLT. Dosages of immunosuppressive drugs were to be tabulated per patient, including start and stop dates and plasma immune levels (if available). Serological and urine analysis parameters were to be tabulated as represented in the study database by patient and visit.

Efficacy variables:
The plasma concentration of ^{13}C urea was to be calculated and presented at baseline and after HHLivC therapy per individual patient.
Number, duration and severity of metabolic crises were to be analysed by patient and overall by descriptive statistics.

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Listings were to be given for laboratory parameters I (ammonia and glutamine). Descriptive statistics for laboratory parameters II (amino acids and blood gases) were to be given for absolute values and differences to baseline by visit and the last available value prior to first OLT. The dosage of ammonia scavenging drugs was to be given as absolute values until the last available value prior to first OLT.

Summary of results

Safety Results:

Surgical placement of a Broviac portal vein catheter was performed in all 12 patients. 6 sessions of liver cell infusion were performed in 10 of 12 patients; in 2 patients therapy had to be discontinued after 1 infusion and 1 partial infusion, respectively, due to catheter dislocation. There was no case of catheter-associated infection or thrombosis, and there were 2 reports of haemorrhage after catheter removal.

The oxygen saturation did not fall below the limit of 90% at any of the timepoints during the application visits V1-V6. Decreases in oxygen saturation were maximum 8% at any time point compared to the baseline of the respective visit. The PVP increased over the threshold defined in the protocol occasionally but these increases were transient and mean values were generally stable and well below 22 mmHg during applications. With respect to oxygen saturation, PVP and PVF, the procedure of HHLivC application can be considered to be safe.

25 adverse events which were judged to be at least possibly related to catheter placement were reported for 11 out of 12 patients. Three of these AEs were serious: Chylous ascites in patient 0103, most probably caused by the injury of a small lymph vessel and device dislocation in patient no. 0106 and 0111. The device dislocation led to premature study discontinuation prior to the second session of HHLivC infusion in both patients.

AEs were analysed as secondary safety variable. All 12 patients (100%) experienced a total of 358 AEs and at least 1 SAE after the first attempt of catheter placement. AEs leading to withdrawal from study/treatment were recorded for 2 patients. In 3 patients, AEs with a possible relationship to treatment as judged by the investigator were recorded.

None of the SAEs was judged to be related to liver cells by the Investigator and by independent sponsor assessment. Eight patients experienced SAEs with possible relationship to immunosuppressive treatments as per sponsor assessment. No trend was visible regarding the AEs and SAEs, all cases were considered not to be related to study treatment and appear to reflect the underlying disease.

Following liver cell therapy, 11 patients received an OLT. Two patients (no. 0102, no. 0104) were stable until OLT, but died due to complications which occurred in the context of the liver transplantation.

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Basic immunosuppressive therapy included CNI (tacrolimus/cyclosporin) in combination with corticosteroids. The doses and levels of immunosuppressive drugs were not different from those in paediatric liver transplantation, except for intraoperative steroid bolus which is not performed for UCD patients in this study.
The data available for safety evaluation in this analysis do not point to any specific or especially unexpected risks due to HHLivC treatment.

Efficacy results:
The number, duration and severity of metabolic crises were evaluated as the main clinical parameter of efficacy. In total 31 hyperammonaemic events with ammonia levels >150 µmol/L within the clinical phase of the study were identified for 10 of 12 patients evaluated in the FAS. Most of these hyperammonaemic events were mild (peak ammonia level <250 µmol/l, n=16 in 8 patients) or moderate (peak ammonia level >250 µmol/l but <500 µmol/l, n=11 in 7 patients). All mild and moderate hyperammonaemic events in this study could be controlled conservatively. In the sensitivity 1 subset (excluding patients no. 0106 and 0111 with incomplete LCT) the total number of crises in 9 out of 10 patients was 29 with an incidence rate of 0.59 (SD=0.73). Mean duration was 3.6 days (SD=2.8)
4 severe metabolic crises with a peak ammonia concentration >500 µmol/l occurred in 2 patients (no. 0103 and 0108, 2 events in each patient), 2 of them were associated with coma of 2.5 days and 19 days. Both children received dialysis on 5 and 1 occasion(s), respectively. These 2 hyperammonaemic events were triggered by external factors.

Among the 5 study patients with ^{13}C ureagenesis results at more than 1 time point, 2 patients showed sustained increase in ^{13}C-urea formation after treatment with HHLivC in terms of peak level as well as AUC in the time course profile of ^{13}C-urea concentration in plasma. These patients were followed for 7 months after HHLivC infusion and received OLT thereafter. It is concluded that this increase in ureagenesis can only be attributed to a sustained engraftment of functional donor hepatocytes into the subject's livers.
In a third patient (no. 0109), the pre-treatment assessment of ureagenesis was not valid due to technical analytical issues, but there are results available at 2 and 4 months after liver cell therapy. Since peak level of ^{13}C-urea plasma concentration and AUC were lower at 4 months in comparison to the 2-month evaluation, it might be concluded that after liver cell infusion there was a transient increase in ureagenesis capacity in this patient which possibly was terminated due to insufficient immunosuppression for intervals of 5–10 days prior to the 4-month evaluation.
In summary, 2 out of 4 study patients with valid pre- and post-treatment results in the ^{13}C-

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ureagenesis assay showed a sustained increase in ^{13}C-urea formation over 7 months after treatment with intraportal liver cell infusion.
AUC (0-120 min) values of all patients with valid pre-treatment baseline and post-treatment measurements were analysed by a paired t-test after log-normalization of data. Only the mean difference of -0.54 between pre-treatment and individual maximum (AUC V-1 – AUC max) was statistically significant at an error level of 5% (p=0.02).

In 6 out of 7 evaluable study subjects at least small quantities of donor cell material were detected by means of PCR and/or immunohistochemical methods several months after LCT and it is therefore assumed that infused liver cells had engrafted in the recipient organs.
Further parameters evaluated as efficacy variables included laboratory parameters (plasma glutamine, blood gases, urea in serum, amino acids in plasma, orotic acid in plasma, etc.), nutritional status, growth and protein intake, and the use of ammonia scavenging drugs. These evaluations revealed no specific influence of liver cell therapy. All patients received natural protein from breast feeding and/or milk from milk powder, as well as a supply of essential amino acids. All patients gained weight and height during the course of the study. All patients received ammonia scavenging drugs per os and i.v. in the course of the study. In addition to scavenger drugs, the patients received arginine and/or citrulline for metabolic stabilization.

Conclusion
In study CCD02, it was possible to successfully bridge children to OLT. 11 of 12 patients received an OLT after a median time of 26.5 weeks after the last infusion of liver cells.

All mild and moderate hyperammonaemic events in this study could be controlled conservatively. Severe metabolic crises during the study were reported for only 2 patients, triggered by external factors.

In total, study CCD02 demonstrated a favourable safety profile of liver cell treatment with respect to catheter placement, intraportal liver cell infusion and overall AE evaluation. The infusion of liver cells can be considered as safe, based on the parameters oxygen saturation, portal vein pressure and portal vein flow. Overall, the procedure of application of the portal vein catheter, infusion of liver cells and immunosuppression was safe, according to the evaluation of AEs. Results of the efficacy evaluation indicate a therapeutic efficacy of HHLivC infusion in children with urea cycle disorders.

Date of report: 22 September 2016