22 October 2015
EMA/CHMP/746584/2015
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Heparesc

International non-proprietary name: human heterologous liver cells

Procedure No. EMEA/H/C/003750/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

AE Adverse event

AP Alkaline phosphatase

APE Atom percent excess

ASLD Argininosuccinate lyase deficiency

ASSD Argininosuccinate synthetase deficiency

ATMP Advanced Therapy medicinal product

BW Body weight

CHOP Childrens`s Hospital of Philadelphia

CMV Cytomegalovirus

CNI Calcineurininhibitor

CPAP Continuous positive airway pressure

CPS1D Carbamyl phosphate sythetase I deficiency

CRF Case report form

CRO Contract Research Organization

CRP C-reactive proteine

CNI Calcineurin inhibitor

CNS Central nervous system

DOL Days of life

EBV Epstein-Barr virus

FAS Full Analysis Set

FISH Fluorescent DNA in situ hybridization

FUV Follow-up Visit

FV Final Visit

GGT Glutamate-glutamyl transferase

GOT/ASA Glutamate-oxaloacetate transferase

GPT/ALA Glutamate-pyruvate transferase

HHLivC Human Heterologous Liver Cells

HIV Human immunodeficiency virus

HLA Human leukocyte antigen

HV Healthy volunteers

IEM Inborn errors of metabolism

IgG/IgM/IgA Immunoglobulines

INR International Normalized Ratio

IMP Investigational medicinal product

ITT Intent to treat

IU International Units

LCI Liver cell infusion

LLOQ Lower limit of quantification

LN Liquid nitrogen

LOCF Last observation carried forward

NAGS N-Acetylglutamate synthetase

NCG N-carbamylglutamate

no. number

OLT orthotopic liver transplantation

OTCD Ornithine Transcarbamylase Deficiency

PCP Pneumocystis pneumonia

PP/RC Per protocol / Relevant control

PVC Portal vein catheter

PVF Portal vein flow

PVP Portal vein pressure

SAE Serious adverse event

SAF Safety Set

SAP Statistical Analysis Plan

SD Standard Deviation or Study Day

SOC Standard of care

SRY Sex determining region of Y

UCD Urea cycle disorder

V Study visits

YSTR Y-chromosomal short tandem repeat

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Cytonet GmbH&Co KG submitted on 5 December 2013 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Heparesc, through the centralised procedure falling within the Article 3(1) and points 1a and 4 of Annex of Regulation (EC) No 726/2004.

Heparesc, was designated as an orphan medicinal product EU/3/10/818 on 17/12/2010. Heparesc was designated as an orphan medicinal product in the following indication: Treatment of citrullinaemia type 1.

Heparesc, was designated as an orphan medicinal product EU/3/07/470 on 14/09/2007. Heparesc was designated as an orphan medicinal product in the following indication: Treatment of ornithine-transcarbamylase deficiency.

Heparesc, was designated as an orphan medicinal product EU/3/10/819 on 17/12/2010. Heparesc was designated as an orphan medicinal product in the following indication: Treatment of hyperargininaemia.

Heparesc, was designated as an orphan medicinal product EU/3/10/820 on 17/12/2010. Heparesc was designated as an orphan medicinal product in the following indication: Treatment of argininosuccinic aciduria.

Heparesc, was designated as an orphan medicinal product EU/3/10/821 on 17/12/2010. Heparesc was designated as an orphan medicinal product in the following indication: Treatment of carbamoyl-phosphate synthase-1 deficiency.

The applicant applied for the following indication:

“Treatment of paediatric patients from birth to less than 6 years of age with urea cycle disorders (UCD) caused by carbamoylphosphate synthetase 1 deficiency, ornithine transcarbamylase deficiency, argininosuccinate synthetase deficiency (citrullinaemia type 1), argininosuccinate lyase deficiency (argininosuccinic aciduria), or arginase deficiency (hyperargininaemia).”

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application composed of administrative information, complete quality data, non-clinical and clinical data based on applicants’ own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies)

The applicant indicated that human heterologous liver cells were considered to be a new active substance.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0250/2013 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP EMEA-000067-PIP02-11-M01 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Applicant's request(s) for consideration

Conditional Marketing Authorisation

The applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14(7) of the above mentioned Regulation and Article 2(3) of Commission Regulation (EC) No 507/2006 based on the following claim(s):

Medicinal products designated as orphan medicinal products in accordance with Article 3 of Regulation (EC) No 141/2000.

New active Substance status

The applicant requested the active substance human heterologous liver cells contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Community.

Protocol Assistance

The applicant received Protocol Assistance from the CHMP on 18 March 2010. The Protocol Assistance pertained to clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Manufacturers

Inspection of the manufacturing sites was carried out by the inspectorate of the responsible authority. The findings of the inspection are in compliance with the EU Good Manufacturing Practice requirements.

1.3. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

CHMP Coordinator (Rapporteur): Jan Müller-Berghaus

CHMP Coordinator (Co-Rapporteur): Pierre Demolis

- The application was received by the EMA on 5 December 2013.
- The procedure started on 26 December 2013.
- The Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 15 March 2014. The Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 17 March 2014.

- During the meeting on 25 April 2014, the CAT agreed on the consolidated List of Questions to be sent to the applicant.
- The applicant submitted the responses to the CAT consolidated List of Questions on 22/10/2015.
- The summary report of the inspection carried out at the following site(s) Cytonet LLC between 12 -15 May 2014 was issued on 08/08/2014.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CAT and CHMP members on 28/11/2014.
- During the CAT meeting on 18/12/2014, the CAT agreed on a list of outstanding issues to be addressed in writing and/or in an oral explanation by the applicant.
- The applicant submitted the responses to the CAT List of Outstanding Issues on 19/02/2015.During the meeting on 12/03/2015, PRAC adopted the PRAC advice
- During the CAT meeting on 16/04/2015, outstanding issues were addressed by the applicant during an oral explanation before the CAT.
- During the meeting on 13/05/2015, the CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a Marketing Authorisation to Heparesc on 13/05/2015.During the meeting on 21/05/2015, the CHMP agreed on a list of outstanding issues to be addresses in an oral explanation by the applicant
- During the CHMP meeting 23/06/2015, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 25/06/2015, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a Marketing Authorisation to Heparesc on 25/06/2015.

1.4. Steps taken for the re-examination procedure

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

CHMP Coordinator (Co-Rapporteur): Andrea Laslop and Ondrej Slanar

- The applicant submitted written notice to the EMA on 09 July 2015 to request a re-examination of Heparesc CHMP opinion of 25 June 2015.
- During its meeting on 21-24 September 2015, the CHMP appointed Ilona Reischl as Rapporteur and Tomáš Boráň as Co-Rapporteur and Andrea Laslop and Ondrej Slanar as CHMP Coordinators.
- The applicant submitted the detailed grounds for the re-examination on 28 August 2015 (Appendix 2 of Final Opinion). The re-examination procedure started on 28 August 2015.
- The Rapporteur's Assessment Report was circulated to all CAT and CHMP members on 29 September 2015. The Co Rapporteur's Assessment Report was circulated to all CAT and CHMP members on 29 September 2015. .
- During a meeting of the Ad Hoc expert group on Heparesc on 6 October 2015, experts were convened to consider the List of Questions for Experts and grounds for re-examination
- The Rapporteurs circulated the Joint Assessment Report on the applicant's detailed grounds for re-examination to all CHMP members on 14 October 2015.

- During the CAT meeting on 15 October 2015, the detailed grounds for re-examination were addressed by the applicant during an oral explanation before the CAT.
- During the meeting on 16 October 2015, the CAT, in the light of the scientific data available and the scientific discussion within the Committee, the CAT re-examined its initial opinion and in its final opinion concluded that the application did not satisfy the criteria for authorisation and did not recommend the granting of the marketing authorisation.
- During the CHMP meeting on 20 October 2015, the detailed grounds for re-examination were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 22 October 2015 the CHMP, in the light of the scientific data available and the scientific discussion within the Committee, re-examined its initial opinion and in its final opinion concluded that the application did not satisfy the criteria for authorisation and did not recommend the granting of the marketing authorisation.

2. Scientific discussion

2.1. Introduction

This application concerns human heterologous liver cells (an ATMP) for the treatment of patients with specific urea cycle disorders (UCD), i.e. carbamoylphosphate synthetase 1 deficiency, ornithine transcarbamylase deficiency, argininosuccinate synthetase deficiency, argininosuccinate lyase deficiency, arginase deficiency. These urea cycle disorders are orphan conditions that predominantly affect children. The clinical course is typically characterised by an initial hyperammonaemic event shortly after birth that also leads to the diagnosis. A sizeable fraction does not survive this initial hyperammonaemic event. It is conceivable that clinical status, especially as regards CNS, and local treatment practises determine how aggressively the further treatment is continued. The clinical course is also dependent on the severity of the defect which is determined by genetic alteration of the respective gene.

The current available treatment regimen are a combination of the avoidance and prophylaxis of catabolic situations, restriction of protein intake, substitution of specific amino acids and ammonia scavenging drugs such as sodium phenylacetate, sodium phenylbutyrate or sodium benzoate. In hyperammonaemic crises dialysis is used to acutely lower ammonia levels. Curative treatment can be achieved by orthotopic liver transplantation (OLT) which can be performed with a reasonable success rate in children from about 10 kg body weight.

Mortality of UCD is high, especially in the neonatal period, and the majority of patients that survive the initial crisis suffer from cerebral damage at one year.

Heparesc is principally intended as bridging therapy until the children are old enough and have reached the appropriate body weight to receive a liver transplant.

HHLivC was classified by the Committee for Advanced Therapies (CAT) and Committee for Medicinal Products for Human Use (CHMP) as a cell therapy medicinal product eligible for centralized approval (EMEA/412541/2005).

Heparesc is presented as a cryopreserved liver cell suspension concentrate containing ≥ 7.5×10^6 cells/mL for re-suspension and subsequent infusion. The cryopreserved product is provided in cryobags with 65 g nominal fill weight with resulting specification of ≥ $487,5 \times 10^6$ total cells. Heparesc is intended to be administered in 6 daily dosages, with dosage calculated on the basis of kg body weight.

At the clinical site, preparation and patient weight specific adjustment of dosage of the cell suspension for infusion is performed. This results in an estimated 8-fold reduction of excipients, when administered to the patient. Additionally, several controls are performed on the reconstituted product.

The application is a complete independent application; the applicant requested a conditional marketing authorisation.

Protocol Assistance regarding the clinical development (use of historic control) was received from the CAT in March 2010.

2.2. Quality aspects

2.2.1. Introduction

Heparesc is a somatic cell therapy medicinal product of allogeneic source. For production, primary human liver cells are isolated from non-transplantable human US donor organs and refined in a manufacturing process, cryopreserved as cell dispersion, and stored in the vapour phase of liquid nitrogen. The product is developed for the support of urea cycle function in patients with inborn liver-based metabolic disorders of urea cycle (UCD) until accessible for liver transplantation. Cells are prepared for intravenous administration. The heterologous cells are applied repeatedly as a single daily dose, adjusted to the body weight of the patient, over a period of maximum six consecutive treatment days via an intraportal catheter. Liver cells administered via the portal vein bloodstream are expected to either penetrate the endothelial layer and integrate into the recipient's liver parenchyma, or be eliminated by phagocytic clearance of remaining donor cells taking place at the surface of blood vessels in the host liver.

2.2.2. Active Substance

General Information

The starting material is US liver organ tissue when declared not suitable for transplantation according to US national standards. Livers are explanted under conditions identical to those implemented for liver organ transplantation and are procured in compliance with Directive 2004/23/EC and the United Network for Organ Sharing (UNOS) guidelines for the USA.

The active substance is produced by Cytonet LLC., Durham, North Carolina, USA. The finished product is imported into the European Union and release testing is performed by Cytonet GmbH & Co. KG, Heidelberg, Germany or authorised contract testing laboratories in Germany. QP release is performed by the Heidelberg site.

One Heparesc batch is derived from one donor organ only. The Applicant implemented a two-tiered system using the Organ Procurement Organisation (OPO)'s unique organ identification number together with a manufacture-identifier based on incoming date. The system is designed to provide unique identification capable to ensure full traceability from donor to product and vice versa.

Manufacture

The manufacturing process of the Human Heterologous Liver Cells (HHLivC) cryopreserved product from liver tissue is one continuous process.

For manufacturing of the active substance, liver cells are isolated from non-transplantable donor organs. The digestion of the liver tissue is achieved by perfusion with a buffer containing protease. Subsequently, the tissue is mechanically disrupted to release the liver cells from the extracellular matrix. The liver cells

are then filtered, washed and suspended in a sterile HEPES-phosphate buffer supplemented with human serum albumin (HSA). Cell number and viability are determined from the pooled cell suspension.

Microbiological safety is ensured during the procedure by various measures.

A batch numbering system was defined for all manufacturing steps (from the liver starting material to the final finished product) to ensure traceability.

For decontamination, the recovered liver tissue is treated with a buffer solution supplemented with specific mixture of antibiotics.

The cumulative reduction of antibiotics in the active substance and finished product was calculated to be more than 1500-fold and 3000-fold, respectively

Specification

Storage of the active substance intermediate is not foreseen as the Applicant justified that cells are fragile at this stage and further rapid processing up to the final cryopreservation step is mandatory. As a consequence, only minimal active substance control testing is performed (total cell count and viability).

Stability

Not applicable for the active substance.

2.2.3. Finished Medicinal Product

The finished product is presented as a concentrated dispersion for infusion with a proposed strength of ≥7.5 x 10^6 HHLivC cells/mL. The cryopreserved cell suspension is provided in CE-marked cryobags as primary packaging. The nominal fill weight of each bag is 65 g. For each preparation one Quality Control (QC) freezing bag is dedicated to microbiological control. Prior to administration, product bags are thawed and reconstituted at the clinical site, considering the dosage to be applied according to each individual patient's body weight.

Pharmaceutical development

The HHLivC finished product is comprised of two main components:

- The active substance (freshly isolated human liver cells in dispersion);

- The cryopreservation solution.

The excipients are listed in Table 1.

Table 1: Excipient in the composition of HHLivC finished product

Sodium chloride
Potassium chloride
Magnesium sulfate heptahydrate
Glucose monohydrate
4-[(2-Hydroxyethyl)-1-piperazine] ethanesulfonic acid
Disodium phosphate dodecahydrate
Dipotassium phosphate
Sodium hydrogen carbonate
Sodium hydroxide

Water for injections
Dimethylsulfoxide
Human albumin solution
Hydroxyethylstarch
Sodium chloride
Sodium hydroxide
Hydrochloric acid
Water for injections

Cryopreservation is a prerequisite for HHLivC, as this is the only way to maintain functional hepatocytes for longer periods. Furthermore, cryopreservation provides time for performing sufficient microbiological testing, and ensures a continuous availability of HHLivC for the treatment of patients.

Excipients for cryopreservation were selected based on experimental studies.

An appropriate physiological buffer was selected in order to maintain physiological osmolality, pH and adequate electrolyte content.

During the development of the manufacturing process for human heterologous liver cells a number of changes have been made. A comparability assessment of product from these process stages has been undertaken, and of critical importance is the comparability between the process used for the clinical studies and the process for manufacture of the material intended for the commercialisation.

The primary packaging of HHLivC finished product consists of single-use freezing bags with integrally attached tubing set and label pouch. The suitability of the container closure system was tested and confirmed with respect to integrity, biocompatibility, extractables, functionality of design and clinical performance. The secondary packaging material consists of metal cassettes.

Adventitious agents

Analysis of historical data shows that a significant number of the donor organs, used for manufacture of HHLivC finished product, present microbiological contamination. The liver is contaminated either *in situ* or during the procurement procedure. The spectrum of microorganisms shows a broad range of contaminants: majority of germs of normal skin flora, mucous membrane and intestinal flora, as well as typical environmental germs. The number of various non-categorised germs was increased between Hannover and Durham. Moreover, a high percentage of batches is contaminated post-thaw (contamination of the starting material with micro-organisms resistant to the antibiotics or contamination during the process).

The Applicant included microbiological testing of the incoming organ transport medium and introduced an antibiosis perfusion step before organs are further processed. For release of the cryopreserved final product, microbiological control testing is performed on the QC bag. If the organ transport medium is found to be contaminated and no microbial growth is detected in QC samples of the final product, the batch may be released only when the contaminants of the transport medium are identified as bacterial and are not resistant to at least one of the four antibiotics used in the antibiosis step. In addition, an antibiogram according to Ph. Eur. 2.6.27 is established. Fungal contamination detected in transport medium leads to rejection of the batch, independent of the final product's microbiological testing result.

The Applicant implemented as microbiological testing a direct inoculation method compliant to Ph. Eur. 2.6.27, in order to replace the previous Bactec blood culture method. For transport of media, the Applicant implemented an improved microbiological testing method largely compliant to the method described in Ph. Eur. 2.6.1.

The Applicant performed a detailed analysis of raw data regarding the level of microbial contamination accepted for organ transplantation as well as the analysis of non-categorised germs contamination and a respective process analysis.

For suitability testing of the QC tests, a broad micro-organism panel was chosen that included essential species recommended in Ph. Eur. Chapter 2.6.1 and 2.6.27. In addition, based on a risk analysis performed by the Applicant, microorganisms frequently detected in donated livers were included. Microbial species potentially relevant for HHLivC were also addressed during analytical method validation.

In relation to the risk of mycoplasma contamination, the Applicant presented a risk assessment with risk mitigation measures and implemented a validated PCR-based mycoplasma assay.

Viral safety of the finished product entirely depends on the adequate testing of the source material and reagents used during the manufacturing process. The Applicant performed a risk assessment which identified virus-specific measures to be implemented. Criteria for the liver donor eligibility were detailed; selection and testing of donors are in compliance with Commission Directive 2006/17/EC; the quality of the materials used during manufacture is demonstrated.

Considering the recent epidemiology of Hepatitis E virus in developed countries including USA, the Applicant was recommended to introduce testing of donors (over 2 years old) for HEV-RNA and to provide information on the testing method and validation report when completed. In addition, the Applicant was recommended to follow the epidemiology of Dengue and Chikungunya virus infections and to consider additional testing or exclusion measures.

Manufacture of the product

For formulation of finished product, the active substance cells are dispersed in a cryopreservation solution composed of HSA, Dimethyl Sulfoxide (DMSO) and Hydroxy Ethyl Starch (HES). The finished product is packaged in freezing bags with integrally attached tubing set and cryopreserved in liquid nitrogen. Aluminium cassettes provide secondary packaging. The product is stored and shipped in liquid nitrogen. Finished product control testing for batch release is conducted on a thawed and washed product sample QC bag and include determination of cell number and viability, hepatocyte identity confirmation, leucocyte impurity measurement including a leukocyte subtype declaration, in vitro urea synthesis for potency, bag integrity, endotoxin, and microbiological control testing.

Product specification

The release specification, according to ICH Q6B, is for the frozen finished product. In order to assess the quality of the frozen product, it is tested immediately after thawing. Additionally, the product is tested after simulating the clinical preparation and at the time (end-point) required for clinical administration. Specifications of the finished product comply with the relevant principles stipulated in the current edition of the European Pharmacopoeia or have been defined on the basis of the manufacturing experience and the quality of the lots that have been manufactured during pharmaceutical development or for the clinical studies.

Stability of the product

The Applicant provided pivotal stability data to support:

- A shelf life of 18 months when the product is stored below -140^{0}C in the vapor phase of liquid nitrogen;

- An in-use shelf life of 90 minutes post-wash for the dispersion for infusion prepared at the clinical site.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Several Major Objections were raised during the assessment. They were adequately addressed by the Applicant.

The Applicant was requested to provide additional information to demonstrate consistency of batches, as different versions of the manufacturing process were used during pivotal clinical trials and for the intended commercial product (Major Objection). With their responses, the Applicant presented a comprehensive updated strategy to address this concern with i) a prospective approach, ii) a retrospective approach and iii) additional data from new batches:

- Prospective approach where viability and total cell count (post-thaw, post-wash, post-wash at 90 min) and potency are compared between process versions 6, 7, 8 and 8E.
- Retrospective analysis of in-process control data on viability and total cell count to demonstrate comparability of process versions 6 to 8.
- Analysis of the leukocyte content of the final product post-thaw with regard to the potential alloreactivity of the lymphocytes (as a portion of the total leukocyte content of each final HHLivC product) within the process comparison. This was conducted using retention samples from all clinically used batches using the new validated leukocyte FACS method able to determine total CD45+ cells, living CD45+ cells, B cells, NK cells, and T cells.

The overall data provided by the Applicant support consistency of batches derived from the different versions of the process which have been used in the pivotal trials or which are intended to be commercially released (with meanwhile further adjustments implemented in the manufacturing process). This Major Objection was considered solved.

There is a considerable intrinsic donor material variability which impacts on quality parameters of the processed cell suspension and the cell yield for further preparation of Heparesc batches. However, this was considered acceptable and issues raised during the evaluation regarding validation on cell count and viability determination were adequately addressed by the Applicant.

The lack of extensive in-process control testing during active substance manufacture was considered justified as the whole processing time is only about six hours. A number of Other Concerns on the process were raised during the assessment, for instance the perfusion flow rate and qualification and control measures for some materials. All of these concerns were adequately addressed.

For the manufacturing of the active substance, a major focus is on donor selection and microbial control of the incoming organ material. Several important concerns were raised on these issues, including the need to further address the initial bioburden of the donor livers and donor acceptance criteria, as well as microbiological controls of the starting material and the final product.

With regard to another Major Objection and several related other concerns, the Applicant developed an updated approach to improve the overall microbiological control strategy by implementation of a microbiological testing, direct inoculation method compliant to Ph. Eur. 2.6.27, in order to replace the current Bactec blood culture method. For transport of media, the Applicant implemented an improved microbiological testing method largely compliant to the method described in Ph. Eur. 2.6.1. In order to reassess the incidence of non-categorised germs and their assumed increase after transfer of manufacturing activities from Hannover to US Durham production site, raw data were submitted to a

microbiologist. Complete re-analysis of raw data was provided. Demonstration that the observed level of contamination will remain at an acceptable level for organ transplantation was addressed in a risk assessment and the corresponding data were provided. The data and justification provided by the Applicant are considered satisfactory and this Major Objection was considered resolved.

Criteria for donor qualification regarding viral safety were generally considered adequate. The Applicant presented data from a risk analysis performed by an external expert in order to address another Major Objection. The risk assessment identified virus-specific measures which resulted in an update of the donor eligibility determination requirements virus testing and donor exclusion measures have been carefully addressed. Most points were satisfactorily addressed. However, considering recent epidemiology, the Applicant was recommended to introduce testing of donors (over 2 years old) for HEV-RNA. The Applicant was also recommended to provide information on the testing method and validation report when completed. The Applicant was also advised to follow the epidemiology of Dengue and Chikungunya virus infections and to consider additional testing or exclusion measures.

With regard to clinical donor eligibility criteria, the Applicant conducted an additional separate analysis of active substance /finished product batch data produced from donors with varying enzyme levels of either AST or ALT, each divided into 3 groups (normal to 3-fold, 3-5 fold, greater than 5-fold). For finished product potency, there appears to be a clear trend for lower potency levels with elevated AST or ALT enzyme levels. The difference between the 3 groups was not statistically significant but this analysis was clearly underpowered. Therefore, although no clear negative impact of donors with high ALT/AST enzyme levels on finished product quality could be found during the review, further analysis using raw values for AST/ALT vs. potency instead of classified values was recommended.

During pharmaceutical development, the Applicant did not consider a possible contamination with mycoplasma, although such contamination may be introduced via the source material, the manufacturing process or the procurement procedure and exposure to personnel. It was considered that the antibiosis step does not sufficiently target potential mycoplasma contamination due to different antibiotic susceptibility profiles. In addition, the microbial control testing regime was not considered suitable to support mycoplasma growth and the sterility test may therefore fail to detect a contamination. In consequence, the lack of a risk evaluation, risk minimisation and effective control measures regarding potential contamination with mycoplasma testing were raised as a Major Objection. The Applicant solved this issue with the implementation of an adequately validated PCR-based mycoplasma assay and the submission of the corresponding risk assessment, which adequately covers all aspects that are critical for a potential contamination risk with mycoplasma. On the basis of all the data provided by the Applicant, it was concluded that the measures in place are adequate to address the risk of transferring mycoplasma with the finished product. This Major Objection was considered solved.

Another Major Objection challenged several aspects of the impurity determination in view of potential alloreactivity, including the acceptance criterion for percentage of remaining leucocytes in HHLivC. Quality control focuses on the finished product and is performed on dedicated containers from each lot after thawing. Beside endotoxin and microbiological control testing, this includes acceptance criterion for CD45+ Leucocyte content (≤20%) as measured by flow cytometry. The Applicant agreed that, in addition to total leukocytes, leukocyte subpopulations should be characterised and quantified in order to specify remaining alloreactive leukocytes injected by kg body weight. A new quantitative single platform flow cytometric method able to determine leukocyte subpopulations/ml (CD45+ CD3+ 7-AAD-, CD45+ CD19+ 7-AAD-, CD45+ CD56+ CD16+ 7-AAD was validated and implemented. Results of these sub-populations were reported by the Applicant.

To address the concern on the acceptance criterion for leukocyte content (≤ 20%), the Applicant proposed a revised acceptance criterion, namely the maximum tolerable amount of remaining leukocytes

due to the change in the method to quantify total leukocytes and leukocyte sub-populations. The specification was intended to be changed from percent to an absolute amount of cells / mL. The Applicant committed to assess a representative number of batches from the current commercial process. Additionally, retrospective assessment of retain samples was foreseen. The gained cumulative data were intended to be used to justify a new acceptance criterion for remaining leukocytes. Additional data and justification of the new limit were provided. Based on the new data, the Applicant revised its previously acceptance criteria of ≤ 20% for total CD45+ leukocytes to < 10 %. As the direct quantification employed in the new assay now reports "cells/µL" instead of "%", the new acceptance criteria was set using the maximum value in the group of clinically used batches in the final product post thaw, namely ≤ 1075 cells/µL. The corresponding finished product acceptance criterion at release was updated; however the stability acceptance criterion was not. This value comes from a batch manufactured according Process #7. Though, there is no statistical difference between Processes 7, 8 and 8E; the data presented show that the leukocyte content in Process 8E batches is more reproducible than in Process 7 batches, suggesting that the commercial process is better controlled. Taking into account the apparent improvement of leukocyte content manufactured according to Process 8E, the Applicant was recommended to further review their limit when additional data will be available with commercial batches. Furthermore, potentially (living) alloreactive cells are below 1%. As the analysis showed that only a minor fraction of total CD45+ leukocytes are potentially alloreactive lymphocytes (T-lymphocytes), these populations should be routinely monitored.

The recently established cell culture assay measuring *de novo* generated urea *in vitro* was accepted as a highly relevant potency assay addressing functional properties of the cells being crucial for their mode of action. The Applicant was asked to elaborate further on any available information on the relevance of this assay for clinical efficacy. Additional clarification was requested with respect to the acceptance limit and the suitability of the test to discriminate subpotent batches. Regarding inclusion of subpotent batches in the validation runs, the Applicant clarified that the urea cycle incompetent liver cell line HepG2 was used. The Applicant also emphasized that the earlier version of the urea potency assay was not adequately validated. Therefore, comparison of historical data from old batches was not considered meaningful. This view was shared, taking into account the history of product development before implementation of the Advanced Therapy Medicinal Products EU Regulation. The justification of the acceptance criterion for the potency assay raised further questions as the parameters for specificity and accuracy of the urea measurement had to be re-validated upon request. To address this, the Applicant analysed the urea spike recovery on 3 batches at different concentration levels for 24 hours and 20 days of sample storage at ≤ -20°C. The minimum and the maximum of spike recovery for 24 hours sample storage time were respectively 84.8% and 111.4%, and for 20 days sample storage time were respectively 85.7% and 103.5%. Therefore, the results of the new validation study justify the acceptance criterion of 80-120% for specificity and accuracy. In summary, 23 batches from Process #8 were used to establish the acceptance criterion for the potency assay, and results of 104 additional batches from Process #8 were documented. Nevertheless, the Applicant did not provide a comprehensive answer about the reasoning to divide the delta C UREA by 2 to calculate the acceptance criterion of the potency assay. Therefore, in the absence of a clear justification of the calculation of the potency acceptance criterion (proposed as 2 µg/1.2x10^6 cells at 180 min), the acceptance criterion should be adjusted to 3.98 µg/1.2 10^6 cells at 180 min corresponding to the minimal value used in clinical trial batches, unless otherwise justified. The Applicant revised the finished product specifications accordingly and the issue is considered resolved.

Another Major Objection was related to the preparation of the ready-to-use cell dispersion for administration at the clinical site directly before administration (i.e. thawing, washing, re-suspension of cells, adjustment of concentration/cell numbers to be administered). An updated description of the preparation procedure, supported by validation data, was presented by the Applicant. This Major

Objection was considered resolved provided the product information is revised accordingly (SmPC section 6.6). In addition, the Applicant explained that no final control is performed after thawing and washing of the liver cells at the clinical site before administration to the patient. After clinical preparation of the product, the concentration of living cells is determined in order to adjust the volume to be transfused to the actual body weight of the patient. The treating physician is in charge of the conduct of the preparation including cell counting as a prerequisite to correct dosing. The issue was considered resolved.

The various updates to the SmPC requested during the procedure have been implemented.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The overall Quality of Heparesc is considered acceptable when used in accordance with the conditions defined in the SmPC. The different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines. The manufacturing process of the active substance and finished product is adequately described, controlled and validated. The quality of the finished product is controlled by adequate test methods and specifications.

Adventitious agents safety including TSE have been sufficiently assured.

However, the following Recommendations were made to the Applicant in case the benefit/risk balance of Heparesc had been positive:

- The Applicant is recommended to introduce testing of donors (over 2 years old) for HEV-RNA and to provide information on the testing method and validation report when completed.
- The Applicant is recommended to follow the epidemiology of Dengue and Chikungunya virus infections and to consider additional testing or exclusion measures for blood or tissue donations.
- The Applicant is recommended to include liver cells of patients suffering from urea cycle defect livers, if available as negative control instead of cell line HepG2, for the control of the finished product potency.
- The Applicant is recommended to further review the limit for leukocyte content when additional batch data is available.
- Although no clear negative impact of donors with high ALT/AST enzyme levels on finished product quality could be found during the review, further analysis using raw values for AST/ALT vs. potency instead of classified values is recommended with increased product experience.
- In relation to control of starting material, the Applicant is recommended to complete section 3.2.S.2.3 with the macroscopic description provided in the biopsy assessment report, summarising the staining results.

2.3. Non-clinical aspects

2.3.1. Introduction

The repeated dose toxicity study and its pilot study (CSP06 and CSP06 pilot), the local tolerance study for HEPES (CPS04) and the acute toxicity studies of three formulations containing HEPES in mice (CPS02) and in rats (CPS03) were performed according to GLP for safety studies.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Despite the existence of a pertinent animal model mimicking one of the intended indications (spf-ash mouse, deficient in ornithine transcarbamylase (OTC)), the applicant decided to select the WWHL rabbit animal model, to demonstrate the proof-of-concept of the treatment HEPARESC. One primary pharmacodynamics study has been realized in the WWHL rabbit (deficient in functional low density lipoprotein (LDL) receptors, and therefore exhibiting elevated serum concentrations of LDL cholesterol) to support the proof-of-concept of the product HEPARESC. Rabbit liver cell preparations were used as test articles.

The LDL-cholesterol serum results obtained throughout the study showed that nine repeated liver cell administrations (9 x 2.5 10^7 cells), infused via intraportal injection, provided a moderate but significant decrease of blood LDL cholesterol levels when compared to the control group. These improvements were maintained in all animals over 120 days after the first cell infusion, and until 12 months in some single animals.

Although the Watanabe model may be able to show that, in principle, a metabolic defect of the liver may be corrected by infusion of allogeneic liver cells, this in vivo study does not mimic specifically the clinical pathology i.e. UCD (urea cycle disorders). This study only supports the general concept of intraportal hepatocytes perfusion, in the case of liver metabolic diseases.

Even if the proof of concept was indirectly established, and because of the weakness of the clinical demonstration of efficacy in patients during the clinical trials, some additional experiments could have been conducted in a more pertinent animal model, such as the spf-ash mouse model, so as to reinforce the proof of concept and support efficacy of HHLivC.

The use of the spf-ash mouse model could also have been useful to determine the optimal doses of hepatocytes to be injected to get a therapeutic effect (dose finding studies). This would have strengthened the determination of the dose to be used in humans. Similarly, this model would have allowed a better analysis of the side effects resulting from injection of large dose of cells in the context of urea cycle disorder. Such data would be supportive for the clinical efficacy study. However, the Applicant also did not perform studies to validate the 13C-assay in small animal UCD models. This was justified by the Applicant based on technical restrictions, i.e. the mice would not contain enough blood needed for the validation procedure. Although this argument appears weak and a technical solution might have been feasible, no further questions were maintained from the non-clinical perspective.

The Applicant used literature data on studies performed in different diseases (Crigler-Najjar-syndrome, phenylketonuria) to choose a dose calculated as 5 % of the cells of the liver. This dose has then been used throughout the studies and upon CAT request for further justification of the chosen dose, animal data in the WHHL rabbit model were presented to support this dosing. The CAT did not request further data for dose finding.

Secondary pharmacodynamic studies

Secondary pharmacology studies were not performed; since secondary pharmacological effects from allogeneic liver cells are not expected, the CAT accepted the omission.

Safety pharmacology programme

Safety pharmacology studies were not performed; the CAT accepted the omission for the same reason.

Pharmacodynamic drug interactions

Not applicable.

2.3.3. Pharmacokinetics

According to the Guideline on Human Cell Based Medicinal Products (EMEA/CAT/410869/2006) conventional pharmacokinetics studies are usually not relevant for human CBMP.

The applicant did not perform a dedicated biodistribution study with the final drug product. Instead, a non-GLP biodistribution study was designed by the Applicant to investigate in NZW and WHHL rabbits the sinusoidal uptake capacity, shunting to the lung, distribution and portal hemodynamic changes, potentially induced by infusion of rabbit hepatocytes or surrogate particles of hepatocytes, composed of macro-albumin aggregates (MAA), labelled with ^{99m} Tc-pertechnetate.

Results revealed, in one animal, that pulmonary shunting could occur even after a first cell dose of 2.5 x 10^7 hepatocytes infused. This event was accompanied with a significant drop in peripheral blood oxygen saturation and transiently induced changes in hemodynamic portal vein flow parameters. For this animal, histological lung samples showed a considerable number of cells in the lung vascular bed. Importantly, there were no additional body compartments to which hepatocyte-like particles distributed after intraportal administration identified from this type of study. Signs of liver damage were observed (elevation of ALAT, ASAT, GLDH and lactate dehydrogenase) ; these were transient, and the liver architecture was not impacted.

Experimental evidence suggests that, although most of the hepatocytes injected after intraportal application are retained in the liver, a small number of cells could enter the systemic circulation and reach the lungs. However, all published work suggest that the cells entering the lungs are rapidly destroyed and fragmented by mechanical stress or non-specific phagocytic activity, within a short-time period of 24 hours.

The Applicant also addressed questions of blood pressure changes and liver damage observed within this study concluding that these changes are not major and only transient. The results of these sub-studies were only provided as peer reviewed publications, which bears the risk of a publication bias. According to the Applicant, although only a part of the raw data are retrievable, from the remaining data and upon interviewing the authors of the studies, there was no evidence of a publication bias.

2.3.4. Toxicology

Single dose toxicity

The applicant did not perform single dose toxicity studies; this is acceptable.

Repeat dose toxicity

The applicant performed two GLP repeat-dose toxicity studies, which were conducted in NZW rabbits.

The objective of the pilot/feasibility study (CPS06-PILOT STUDY) was the implementation of the surgical procedure for the implantation of portal vein catheters with subcutaneous (s.c.) vascular access ports (VAP) in rabbits. The second objective was the implementation of the procedure for infusion of liver cells into the portal vein through the catheter and to test the tolerability of different application volumes and cell doses.

In the second and pivotal GLP study (CPS06 STUDY), the purpose was to obtain information on the toxicity of allogeneic liver cells (RHHLivC) in New Zealand White (NZW) rabbits given as repeated daily i.v. infusion (in association with tacrolimus) into previously cannulated portal vein over 6 consecutive days, followed by a 2-week recovery period. The study design mimicked as close as possible the clinical situation. The composition of the test solution was comparable to the human final product except for the human liver cells, which were replaced by rabbit liver cells; human serum albumin, which was replaced by rabbit serum albumin; and collagenase used during the manufacturing of the cell suspension, which was replaced by the collagenase identified most suitable for preparation of rabbit liver cells.

The low number of animals included in each study (5 animals for the study CPS06-PILOT, 32 for the second) was noted and made the interpretation of the observed findings difficult.

Generally, the same findings were reported in both studies. The observations gained during these studies revealed that the surgical procedure was well tolerated, with only typical post-operative symptoms (fibrosis, seromas). No major variations in arterial oxygen saturation or body temperature were observed during the treatment period. During treatment, the heart rate was transiently and moderately increased in single male and female animals of treated groups.

Generally, clinical observations during the infusion (signs of apathy, heart rate alterations, hyperventilation and signs of stress) were observed in both genders. They were more pronounced in the high dose group, and seemed to increase in intensity along the infusion time. Female animals seemed to develop clinical symptoms in a higher frequency and higher intensity when compared to males. The observed hyperventilation and apathy were reported in both studies, and were therefore expected to occur based on literature stating that hyperventilation would be a compensation process to the reported shunting phenomenon (embolization of pulmonary capillaries).

The liver and the lungs were identified as primary target organs of toxicity. The observed macroscopic findings as well as alterations in liver enzymes were expected and are possible related to the infusion of liver cells inducing sinusoidal embolization and ischemia. Ascites was observed in all treated male and female animals at the end of treatment and at the end of the recovery period in both control and treated animals. Even if this event is considered to be not drug-related, the treatment with liver cells could have enhanced this phenomenon since ascites was more frequently observed in the test item group. Nevertheless, portal hypertension is presumed to be the cause and could possibly have been induced by the presence of the intraportal cannulation as well as by a putative partial portal congestion and/or occlusion of sinusoidal spaces with cell, resulting in hemodynamic alterations.

Microscopic liver findings were seen in one male animal of the acute low dose group and one male animal of the acute high dose group as well as one female animal of the acute high dose group and one female animal of the recovery high dose group and might probably be allocated to the treatment with test item.

Finally, a NOAEL was not determined in this study as mild clinical findings (signs of apathy, heart rate alterations and hyperventilation), effects on some haematological parameters (red blood cell system), on some liver enzymes (ALAT, ASAT and AP) as well as macroscopic and histological findings were observed also in the low dose groups.

Genotoxicity

Conventional genotoxicity, carcinogenicity, and reproduction toxicity studies are not required due to the nature of the product (not cultured primary cells), and absence of carcinogenic/mutagenic component used during the manufacturing process.

Considering the nature of the product (allogeneic human cells), the risk of transmitting tumour cells along with the drug product infusion, even if minimal, cannot be completely ruled out, despite the biological and clinical selection of liver donors; HHLivC could contain tumour cells which could proliferate at ectopic sites, leading to tumour formation. However, tumour induction by transplantation of primary liver cells has not been reported irrespectively of whether the cells were given into the liver or injected into ectopic sites such as spleen, peritoneum, the renal capsule, lungs, subcutaneous, skeletal muscle, and various glands.

A concern was raised about possible risk of genotoxic potential for the chemical impurities of the component HEPES, as these impurities were not characterised. The Applicant`s response listed the main impurities are ammonium and ethanol, which are not genotoxic, as well as iron and heavy metal ions in traces that are below the toxicologically defined threshold. Therefore, the possible risk of genotoxicity due to HEPES impurities is considered negligible.

Carcinogenicity

See above.

Reproduction Toxicity

See above.

Toxicokinetic data

Not applicable

Local Tolerance

Local tolerance of Heparesc was evaluated as part of the repeated dose toxicity studies. Results obtained revealed post-operative symptoms (fibrosis, seromas) related to the surgical mode of administration, which also occurred in the control group, and thereby do not raise any safety concern.

Other toxicity studies

During the PIP procedure, the necessity for conducting further non-clinical studies in juvenile animals was considered, so as to address the aspects related to the possible effects of hepatocytes infusion on children lung development, hepatocyte survival time in lungs and elimination from lungs. Such studies imply important technical constraints and have been considered of limited relevance for the administration of HHLivC to paediatric patients, considering the fact that they would not add significant new information to the extensive knowledge that is already available. In 2012, the EMA/PDCO agreed that no non-clinical studies on juvenile animals were needed to support the paediatric condition of treatment of urea cycle disorders.

As the buffering agent HEPES is not approved for i.v. use in humans, the applicant focused on the safety of the injection of the component HEPES, via the realization of three GLP acute toxicity studies (CPS02, CPS03, CPS04) with escalating doses of HEPES. These studies did not show major findings up to a dose of 540 mg HEPES/Kg. For the DMSO, widely used in routine for the cryopreservation of human blood stem cells, toxicity of a final concentration of 5% (v/v) of DMSO was addressed in the same studies, showing the absence of concern related to DMSO. It is underlined that the final concentration of DMSO in the final cell preparation of HHLivC, after washing, is planned to be <1%, which is an acceptable concentration, regarding safety.

2.3.5. Ecotoxicity / environmental risk assessment

No environmental studies (Phase I or III) have been performed.

This was acceptable as the medicinal product Heparesc does not pose a significant risk to the environment, due to its nature, and its biodistribution profile.

2.3.6. Discussion on the non-clinical aspects

Local damage and bleeding, impaired portal blood flow, shunting of liver cells towards the lung capillaries, and immunological reactions are the main safety concerns in the context of HEPARESC, an allogeneic cellular product, for intraportal liver cell infusion.

In the GLP repeated-dose toxicity study in which rabbits were exposed to a suspension of allogenic liver cells once daily for 6 consecutive days by infusion into the liver portal vein, the lungs and the red blood cell system were identified as targets for toxicological surveillance in patients.

Portal embolism, leading to portal hypertension, decreased portal flow, and possibly portal thrombosis, together with shunting of the infused liver cells to the lung are critical and were observed to cause 4 cases of death, out of 107 animals studied in the total of the Cytonet studies in rabbits CPS01, CPS05 and CPS06 together with the other supportive studies from third parties in rabbits (Wilson, Chowdhury et al. 1990; Eguchi, Rozga et al. 1996), dogs (Kay, Baley et al. 1992; Grossman, Wilson et al. 1993; Kocken, Borel Rinkes et al. 1996), pigs (Muraca, Neri et al. 2002; Maruyama, Totsugawa et al. 2003).

While transient decrease in portal blood flow associated with increased portal pressure was observed throughout the studies mentioned, complications arose especially from the infusion of large cell numbers. Muraca *et al.* demonstrated that the effects are in linear relationship to the amount of cells infused (Muraca, Neri et al. 2002).

Experimental evidence suggests that adult hepatocytes are retained in the sinusoids because of their larger size and higher rigidity compared to blood cells. Although most of the hepatocytes after intraportal application will be retained in the liver, small numbers of cells will enter the systemic circulation and reach the lungs. All experimental evidence suggests that the cells entering the lungs are destroyed within 24 hours and are not harmful to the treated subject (Rajvanshi, Fabrega et al. 1999). In the absence of nutrients and growth factors hepatocytes can only survive for a limited period of time.

The CHMP endorse the CAT discussion on the non-clinical aspects as described above.

2.3.7. Conclusion on the non-clinical aspects

From a non-clinical point of view no objections have been raised; concerns highlighted during the non-clinical assessment (including the limitation of the rabbit model) were considered resolved by the CAT.

The CHMP endorse the CAT conclusions on the non-clinical aspects as described above

2.4. Clinical aspects

2.4.1. Introduction

Protocol Assistance regarding the clinical development was received from the CHMP, which was generally accepting the development plan of the company, more specifically the use of a historic control because of the low incidence of UCD. It was mentioned that matching historical controls at inclusion in the study

could be useful. However, the difficulties in interpretation in such a trial were highlighted and it was felt that within patient comparisons would only be acceptable if the natural history of patients could clearly be established. A primary outcome measure consisting of the combination of 13C ureagenesis results and frequency and severity of metabolic crises was considered acceptable in case consistent overall survival was observed.

The originally claimed indication was for: “Metabolic stabilisation of patients with urea cycle diseases.”

Urea cycle diseases are caused by inherited rare deficiencies of enzymes involved in the urea cycle - carbamoylphosphate synthetase 1 (CPS1), ornithine transcarbamylase (OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL), arginase and N-acetylglutamate synthetase (NAGS). However, the applicant obtained orphan designation only for the treatment of ornithine-transcarbamylase deficiency (EU/3/07/470), citrullinaemia type 1 (EU/3/10/818), hyperargininaemia (EU/3/10/819), argininosuccinic aciduria (EU/3/10/820), carbamoyl-phosphate synthase-1 deficiency (EU/3/10/821). Thus, the OD for NAGS deficiency is missing.

Therefore, the applicant amended the indication to reflect the enzyme deficiencies covered by the applicant`s orphan designations, and to clarify that Heparesc is intended as a bridging treatment until OLT is possible. The final claimed indication was for:

“Treatment of paediatric patients from birth to less than 3 years of age suffering from severe urea cycle disorders (UCD) for whom orthotopic liver transplantation is considered a treatment option at a later timepoint.

Underlying enzyme defects may be carbamoylphosphate synthetase 1 deficiency, ornithine transcarbamylase deficiency, argininosuccinate synthetase deficiency (citrullinaemia type 1), argininosuccinate lyase deficiency (argininosuccinic aciduria), or arginase deficiency (hyperargininaemia). “

Patients treated in the main clinical studies represented CPS1D, OTCD, ASSD.

The applicant requested a conditional approval based on the following claims presented by the applicant: demonstrated favourable benefit/risk balance on the basis of interim data from 19 treated patients, life-threatening orphan disease, feasibility to submit further data in the ongoing studies and immediate availability of the medicinal product outweighs remaining uncertainties regarding inherent risks. The ongoing studies CCD02 (EU) and CCD05 (US, Canada) will continue and recruitment is not hampered by immediate availability because all patients in the trial will receive the treatment as there is no concomitant control group.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

However, during the assessment a request for a routine GCP inspection was adopted for the clinical study CCD02, at the sponsor site and the investigator site. The inspection revealed critical and major findings related to study design, conduct and oversight of the study, safety reporting, IMP handling, monitoring of the trial and informed consent handling. The inspection report concluded that the conduct of the trial was GCP non-compliant and recommended the data collected should not be used in the context of the marketing authorisation application. The inspection findings were thoroughly discussed at the CAT meeting in December 2014 and the majority of CAT members were of the opinion that in the context of

the specific disease and the specific intervention, an evaluation of benefit/risk balance is still feasible, provided that the GCP inspection findings are carefully taken into consideration during the evaluation.

Tabular overview of clinical studies

Study ID	No. of study centres / locations	Design	Study Posology	Study Objective	Subjs by arm entered	Duration	Gender M/F Median Age	Diagnosis Incl. criteria	Primary Endpoint
CCD02	/ Germany	Single arm, uncontrolled open, prospective, , multicenter	Viable HHLivC per session a: ≤10 kg: 0.05 x 10^9 per kg BW >10 to 15 kg: 0.5 x 10^9 nonadjusted to BW >15 kg: 0.033 x 10^9 per kg BW. 6 portal vein application sessions on 6 consecutive days.	Safety, Efficacy	12	21 months or 3 months after OLT		<6 years OTCD, CPS1D, ASSD	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
CCD05	/ USA, Canada	Single arm, uncontrolled, open, prospective, multicenter	Identical to CCD02	Safety, Efficacy	8	18 months or 3 months after OLT		<6 years OTCD, CPS1D, ASSD	Changes in ^{13}C-urea formation from baseline to 2 and 4 months (or earlier if OLT is performed prior to month 4 [V19]) after first HHLivC infusion
CCD10	/ Europe	Retrospective, non-interventional, multicentre historical control	N/A	historical control	63	N/A		Neonatal onset OTCD, CPS1D, ASSD	• Hyperammonaemic events • Events of increased glutamine (≥1000 µmol/l) • Severe metabolic crises • Survival
CCD09		Open prospective, diagnostic, multicenter	^{13}C-acetate 55 mg/kg BW	Investigation of urea generation by use of ureagenesis assay	37/37			Healthy, patients with UCS and carriers of UCD mutations	

2.4.2. Pharmacokinetics

Absorption

Conventional absorption, distribution, metabolism, and elimination (ADME) studies are usually not feasible or not relevant for human cell based medicinal products (CAT/EMA “Guideline on human cell-based medicinal products” [EMEA/CAT/410869/2006]). Accordingly PK studies with HHLivC were not performed in humans.

Distribution

No dedicated studies of the distribution of HHLivC were performed. Non-clinical studies and clinical information from similar products support the view that liver cells administered in the portal vein are trapped in the liver and are either incorporated or die e.g. through immune response and are eliminated by phagocytic cells.

In order to demonstrate engraftment and persistence of the cells several methods were developed with the intent to investigate engraftment and perform quantitation of HHLivC in the explanted recipient liver at the time of orthotopic liver transplantation. The choice of method depended on the enzyme affected, the specific mutation and clinical circumstances (i.e. gender mismatch transplantation). Some samples were investigated using different methods. The following methods were investigated:

-Immunohistochemistry against wild type proteins (CPS1, OTC, ASS1)

-Gender specific PCR in the case of male donor and female recipient

-Detection of wild-type donor DNA

-Quantification of wild-type donor DNA

Of the 19 patients that had received HHLivC, 14 patients subsequently received an orthotopic liver organ transplant. Two patients dropped out after 1 infusion and one patient had no available samples. Of those 11 patients that had available material 1 was investigated with a method that proved not suitable for the detection of donor cells. From the remaining 10 patients there was detection of donor cells in 9 patients. Overall the data were difficult to interpret and not quantitative, there was also the possibility that DNA from dead cells was amplified. The most promising method appeared to be digital PCR. Detection was not uniform in different samples which may indicate heterogeneous distribution within the livers. There appeared to be a trend in diminishing positivity over time which could indicate that long term persistence was not obtained.

Elimination

Not applicable.

Dose proportionality and time dependencies

Not applicable.

Special populations

The target population consists of paediatric patients only.

Pharmacokinetic interaction studies

Not applicable.

Pharmacokinetics using human biomaterials

Not applicable.

2.4.3. Pharmacodynamics

Mechanism of action

The physiological role of the urea cycle is to convert toxic ammonia to non-toxic urea that is excreted in the urine. This cycle is defective in patients with urea cycle disorders, which results in hyperammonaemic crises. Heparesc consists of liver cells of allogeneic origin, which thus provide the deficient enzyme activity into the patient`s liver.

Primary and Secondary pharmacology

A 13C-ureagenesis assay was established to document engraftment of functional hepatocytes in the liver of the recipients after HHLivC therapy.

Two pharmacodynamics studies have been performed to characterize the 13C-ureagenesis assay as biomarker for ureagenesis capacity. One study included healthy volunteers (CCD07) and the other study was in healthy volunteers, patients and asymptomatic carriers of UCD mutations (CCD09). The 13C-ureagenesis assay readout is used as efficacy endpoint in the pivotal clinical studies CCD02 and CCD05 in UCD patients.

Method: In both PD studies, subjects received an oral solution of water with sodium [1-13C]-acetate. Plasma and breath samples were taken before and at 10, 20, 30, 45, 60, 90, 120, 150, 180, and 240 min thereafter. These were analyzed for 13C-bicarbonate, 13C-urea and 13CO2 (latter sampled from breath) by Infai (certified accredited applicant). 13C-bicarbonate was included since it is rapidly formed from sodium [1,2-13C]- acetate and is the direct precursor for 13C-urea formation in the urea cycle. The formation of 13C-urea was measured by 13C-isotope ratio mass spectrometry as previously described by (Tuchman Caldovic et al. 2008). Briefly, plasma was incubated with urease to convert urea to CO2; this was introduced into the IRMS analyzer, where the ratio of 13CO2/12CO2 was determined in comparison to a standard reference CO2 sample. Based on the result produced by the IRMS instrument (ratio of 13CO2/12CO2 expressed as δ value) and the total plasma urea concentration, 13C-urea plasma concentrations were calculated using standard formulae. In a final step the natural abundance of 13C-urea (approx. 1%) was eliminated by subtracting the baseline value. Plasma bicarbonate was converted to CO2 by acidification, and 13C-bicarbonate was determined as described above, using the total plasma bicarbonate concentration as reference. 13CO2 in exhaled air was determined directly and expressed as atom percent excess (APE) using standard formulae (Brenna, Corso et al. 1997). In addition, each blood sample was analyzed for total urea and bicarbonate by a central laboratory.

Assay validation: After initial lack of data on precision, accuracy and sensitivity, and poor and confusing documentation of the validation with respect to the quantitative determinations of 13C-urea and 13C-bicarbonate in plasma, the applicant submitted two newly prepared validation reports together with information clarifying which method was used in each of the trials. It transpired that the quantitative 13C-ureagenesis assay for subject samples from Cytonet's clinical studies has been performed at two different laboratories, at the Children`s Hospital of Philadelphia (CHOP) laboratory ("reference method") for the study samples of CCD02, CCD05, CCD07, and at INFAI ("new method") for the study samples of CCD09.

The assessment of the newly submitted validation results led to the conclusion that the validity of the results of the absolute 13C urea determinations in plasma in Cytonet's study samples could not be fully confirmed. The "new method" at INFAI was not fully validated according to the recommendations set by the EMA guideline on bioanalytical method validation for quantitative concentration determinations in study samples from human studies (2011) and the observed average accuracy of 75.9% was too low to meet the acceptance criteria of 85 to 115% accuracy set by the guideline. On the other hand, the CHOP method was now presented as successfully validated, showing good accuracy (101.3%) and precision (4-8 %), and is considered more reliable than the INFAI method. However, it became obvious that at the time of measurement of the study samples of CCD02 and CCD05 (Feb-Mar 2011) no quality assurance system was in place and only partial GLP requirements were fulfilled at CHOP.

Cross validation results of study plasma samples determined with both methods confirmed that the INFAI method yields systematically lower APE results (and thus lower 13C- urea plasma levels) than the CHOP method (consistent with the accuracy bias found for the INFAI method). Nevertheless, cross validation accuracy results were within the 20% limit set by the EMA guideline.

The initially missing descriptions of other quantitative determinations in air and plasma samples (e.g. 13-CO2 in breath, 13C-bicarbonate in plasma via conversion to CO2 by acidification, total urea/bicarbonate measurements in plasma) and their respective validation data were also provided on request. It could be clarified that, as for the determination of 13C/12C ratio from urea in plasma (see above), the determination of total urea in plasma and of 13C-Bicarbonate in plasma by IRMS was performed at the CHOP laboratory for studies CCD02, CCD05, and CCD07, and at INFAI for study CCD09.

Both methods of total urea determinations and the INFAI method for 13CO2 in breath can be regarded as validated. But the validity of the results of the absolute 13C bicarbonate determinations in plasma in Cytonet's study samples cannot be confirmed. The method at CHOP has been validated after measurement of the study samples, and for the INFAI method "the necessity of a full validation was not realized and not performed at the time of the study".

In summary, both the quantitative determinations of the absolute 13C-urea and 13C-bicarbonate concentrations in plasma were not sufficiently validated at the time of measurement of the study samples. Due to a systematic underestimation by the INFAI method, the absolute results of study CCD09 (the study where the INFAI methods were used) should be compared with caution to the study data from CCD07, CCD02 and CCD05. The relative changes observed in a single patient before and after treatment with HHLivC within studies CCD02 and CCD05 (determined by the CHOP method) can be assumed to be more reliable.

The objective of the first study (CCD07) in healthy volunteers (HV) was to evaluate the feasibility and reproducibility of the assay. The use of the C13-ureagenesis assay has been previously described in the literature, providing results of 17 healthy subjects. Therefore, it is reasonable to utilize the same dose of tracer, which was done in study CCD07, and to evaluate the same tracer PK endpoints (e.g. atom % excess (APE) and absolute concentration of C13-urea in plasma and 13CO2 in breath) as in the original method description.

The objective of the second study (CCD09) was to investigate the performance of the urea cycle in the study population of HV, symptomatic patients with UCD and asymptomatic carrier of the ureagenesis enzyme defect. The Applicant's claims related to the study results are (1) AUC of 13C-urea is the most appropriate parameter for determination of the performance of the urea cycle using the 13C-ureagenesis assay; (2) the 13C-ureagenesis assay is a valid biomarker of the performance of the urea cycle and its outcome; (3) together with other clinical parameters, the assay is a useful tool to determine the disease severity of UCD patients; (4) the assay provides a reliable tool to follow changes in ureagenesis capacity and can be helpful in monitoring of success of curative therapies in UCD; (5) the assay is independent of the administered standard treatments.

Endpoints in both PD studies were similar. The Applicant decided on using AUC(2h) and AUC(4h) (primary Endpoints), Cmax and tmax of C13-urea (secondary endpoints) to numerically describe the C13-PK profile. No rationale has been provided for this choice. Also, no attempt had been made to understand the varying plasma C13-urea profiles observed in the studies between HV, asymptomatic carriers and symptomatic patients. The Applicant was therefore asked to discuss in details (a) what influences the shape of the PK profile and the PK parameter derived from it other than disease severity, and (b) how in the studies it was controlled and/or accounted for those influences.

The question concerned the interpretation of the assay readouts derived from symptomatic patients and carriers in comparison to healthy subjects with the intention to decipher if the assay could be useful (reliable and sensitive) for evaluating efficacy of therapeutic intervention over time.

In their answer the Company referred to a model, which was developed based on source data from healthy volunteers, asymptomatic carriers and symptomatic patients of study CCD09. This model was in September 2014 further used to predict C13 urea and C13 bicarbonate plasma concentration for the individual treated patients (study CCD02 and CCD05) to compare them with the actual measured values at baseline and post-treatment. It represented a purely mathematically approach to fit the measured CCD09 data. Its structure was not based on a physiological analogue. The model best described the data, when two ureagenesis pathways were *assumed* – which were labeled as ER (= Ko * tlag) and K2. The Company hypothesized that ER (early urea release) may result from the first pass effect, while K2 describes the formation of urea from the systemic C13-bicarbonate. While this appears an interesting proposal, it would however need to be scientifically proven. A critical observation is that the model has been developed by mathematically fitting a curve to the measured data set only. It is therefore not surprising that the model reflects quite nicely the measured 13C urea and 13C bicarbonate profile over 2 hours of those data. However, the model is not mechanistic. It has not been validated for its predictive power and furthermore is not indicated for performance of simulations. Thus, overall this approach does not provide more insight to the interpretation of the assay readout.

The only studies in treated patients, which so far provide C13 ureagenesis assay readouts to monitor treatment effects, are CCD02 (n=6) and CCD05 (n=8). The profiles of the C13 urea curves differ from healthy volunteers and carriers from study CCD09 by only showing an initial peak instead of a partially reduced and prolonged incline. As this profile is comparable to the symptomatic patient in CCD09, the same unanswered questions remain about the validity of the speculated explanation (ER) for it. The assay readout of the treated patients was not linked to clinical parameters of efficacy. For those three patients, where long term follow-up (15-24 months) is available, the assay readouts are not consistent over time, which either points at uncertainties related to the assay or lack of a stable therapeutic effect.

To support the use of AUC(0-t) as potential surrogate marker for ureagenesis, the Company provided a graph to demonstrate a difference between the three subject groups of CCD09. This graph does not depict the high inter-individual variability (e.g. SD). Albeit significant differences in AUC(2h), AUC(4h), Cmax and tmax were reported between symptomatic patients (AUC(4h): 631 ± 639 SD) and HV (AUC(4h): 2239 ± 448 SD) or asymptomatic patients (AUC(4h): 1880±819), it is noted that the standard deviation varies substantially between groups so that the appropriateness of a t-test for statistical comparison needs to be questioned. At the same time, it appears that these parameters are not useful to differentiate between HV and asymptomatic patients. This raises the question how sensitive the assay parameters are to moderate differences in disease severity, which has been addressed separately (see below).

In summary, the CAT considered that neither the model parameters (e.g. ER, K2) nor kinetic measures (AUC, Cmax, tmax) of the urea profile, which still can`t be fully explained, can serve as reliable surrogate markers to quantify hepatic ureagenesis.

CCD07 in adult healthy volunteers: The study was designed as an open-label, mono-centre, randomized, 2-period, non-controlled observation study with 2 study groups. One group fasted 4 hours

before isotope administration, the other 10 hours. No significant difference was described. 11 subjects received two doses of sodium [1-13C]-acetate (d2 and d15; 27 mg/kg each). Also, no obvious difference with regard to first and second administration was detected. It needs to be noted however, that slight differences in the mean 13C-urea concentrations were seen between groups and with time. These are likely negligible given the wide range of variability. Overall, it is difficult to draw definite conclusions on repeatability from these data.

The results of study CCD07 in healthy volunteers were not consistent with the publication, from which the methodology was adopted. The rate of C13-urea enrichment in plasma did not quite parallel the rise of 13CO2 in breath during the initial phase after oral administration. This is surprising, since > 99% of the administered C13 label is eliminated by breath and the remaining fraction can be considered a precursor for ureagenesis and occurs in C13-urea. In healthy subjects a parallel rise has been described (Tuchman 2008). Furthermore, the rationale to use a C13-plasma profile to reflect on ureagenesis performance was in the paper by Tuchman supported by the fact that shortly after isotope application a plateau was reached suggesting that C13 application was effectively labelling ureagenesis and the plateau may be indicative of the steady state ureagenesis rate. In the current data set the plasma plateau was reached consistently at a later time point (2-4 hours) or not at all, and the maximum C13-CO2 in breath (marking the peak of the precursor availability) occured also delayed (between 45- 60 minutes). This was consistent with the results in healthy volunteers found in study CCD09. Also, total body urea synthesis rate was far below what was reported by Tuchman, and in a range, which has been reported for subjects on a low protein diet (Young 2000). The Applicant was asked to comment on the cause of these discrepancies, and how it influences the reliability and repeatability of the method. A definite explanation for the discrepancy could not be provided. Uncertainties remain with regard to the interpretation of the ureagenesis assay readout.

CCD09 in healthy volunteers, symptomatic UCD patients and asymptomatic carrier: The study was designed as an open, prospective, diagnostic study in 4 study centres. It enrolled HV (n=10, age range 27-53 years), symptomatic UCD patients with genetically confirmed UC gene mutations (n=18, age range 2-32), and asymptomatic carriers of genetically confirmed UC gene mutations (n=9, age range 4-45 years).

Symptomatic patients with confirmed mutation had either confirmed OTCD (4 neonatal onset, 7 late onset) ASSD (5 neonatal onset) or ASLD (2 neonatal onset). Four patients received dialysis at the initial event, several had repeating events. However the patients appeared to be reasonably well controlled with conservative therapy and protein restriction. Five patients were adult, 4 adolescent and 9 were children. Four patients were below 5 years of age corresponding to the inclusion criterion of the CCD02 and CCD05 study, three of these patients had a neonatal onset, one received initial dialysis. In summary these patients appear to be less severely affected than the population included in the HHLivC studies CCD02 and CCD05.

Subjects received one single dose of sodium [1,2-13C]-acetate as oral solution or via a nasogastric tube (NGT) or percutaneous endoscopic gastrostomy (PEG) tube. The dose was twice as high as the one used in study CCD07 and the acetate was double labelled (two C13 per molecule of acetate instead on one). Together this results in a four-fold increase of C13 exposure. Since the recovery of C13 depends on the position of C13 in the acetate molecule, the overall resulting increase of C13 in the bicarbonate molecule (feeding into the ureagenesis pathway and being the only precursor to C13-urea) is 3.3-fold, which explains the 3-times higher Cmax of C13-urea and APE in studies CCD09, 02 and 05. Endpoints and analysis of pharmacokinetic parameters were similar to study CD07.

With regard to the C13-urea PK profile, the same concerns as addressed above applied to the results of this study. In addition, the interpretation of Cmax and tmax was hampered by the fact, that not for all subjects the true maximum value was reached in the observation time and censoring was applied.

An important aspect of this study was the fact that the three groups are intended to serve as representation of different disease severities, since no external marker of disease severity was used to validate the assay readout. Mean total urea levels may serve theoretically for “internal” validation of assay readout, but since there was no clear difference of this parameter between healthy subjects, asymptomatic carriers and patients (due to high standard deviations) its use is not further supported. The inhomogeneity of both groups (symptomatic and asymptomatic carriers being comprised of small subgroups with different enzyme defects) may have contributed to the variability. With regard to external validation, the Company discussed that there is currently no “gold standard” clinical severity score, which would not be influenced by a variety of other factors.

A trend in post-hoc analyses differences in assay readouts was seen between neonatal versus late onset disease and also between subjects with ASSD versus OTCD (possibly missing significance only due to the small sample size, n=3-5). However, those differences were not correlated to a marker of disease severity, leaving the interpretation of the observations open. They may therefore have been caused by differences in disease severity, but could also have been related to methodological errors, or both. Baseline values of ammonia in plasma, which could serve as indicator of disease severity, did not correlate with the assay outcome. The use of concomitant medication may have influenced this relationship. The only “external” indicator for disease severity, which actually correlated with assay readout, was the previous use of ammonia scavenging drugs in symptomatic patients (e.g. AUC(4h), p=0.02; n=18). Furthermore, in a subgroup of symptomatic patients (with ASSD, n=5) an increased baseline value of ammonia in plasma was negatively correlated to C13-urea AUC (-0.97, p=0.0065).

The lack of an “external” validation parameter remains a central problem of biomarker assay validation.

As discussed above, significant differences in AUC(2h), AUC(4h), Cmax and tmax were observed between symptomatic patients (AUC(4h): 631 ± 639 SD) and HV (AUC(4h): 2239 ± 448 SD) or asymptomatic patients (AUC(4h): 1880±819). At the same time, it appears that these parameters are not useful to differentiate between HV and asymptomatic patients. This raises the question how sensitive the assay parameters are to moderate differences in disease severity. In addition, it is noted that the standard deviation varies substantially between groups so that the appropriateness of a t-test for statistical comparison needs to be questioned.

It also should be kept in mind that the observation of a significant difference in the assay parameters between healthy subjects and symptomatic patients in the current study does not inform about the potential of the assay to detect moderate improvement/worsening of disease severity in response to therapy of a patient population with more severe disease and overall minimal ureagenesis capacity. The risk may be that C13 enrichment of the ureagenesis cycle is slowed down, C13 enters alternative pathways or is eliminated through breath, and a sufficient level to label ureagenesis performance is not reached. Such patient population was included in the clinical trials CCD02 and CCD05. The C13 PK profile observed there differed quite substantially from the one observed in the PD study in symptomatic patients. The applicant was asked to elaborate on the interpretation of the different C13 plasma profiles.

This question aimed to understand how alternative metabolic pathways may in healthy and diseased subjects differentially interfere with the C13 precursor availability, subsequently influencing assay readout especially in patients with severe disease. The Company argued that it is unlikely that the first pass ureagenesis would not be impacted by an alternative pathway. This is agreed to, however the role of this pathway for assay readout is only speculative. In addition, alternative pathways/elimination of systemic C13-bicarbonate have not been addressed. Their influence on assay performance has therefore also not been quantified.

To further support their statement, that in the low enzyme activity range (e.g. in patients with severe disease manifestation) differentiation with the assay readout is possible and detection of moderate changes should be feasible, the Applicant referred to C13-urea AUC of nine individuals from study CCD02,

CCD05 and CCD09. This rather anecdotal information did unfortunately not allow to identify a clear response pattern. To link the observed ureagenesis readout with disease entity (enzyme mutation), late-vs early onset and gender the small population was broken down to subgroups of one or two. The dataset was too small to draw any reliable conclusions from or to conclude that assay performance would be sufficiently documented to detect moderate improvement/worsening on this basis.

In summary, assay performance with regard to detection of moderate improvement/worsening in patients with severe disease was not sufficiently demonstrated.

The relationship of C13-urea profile to standard therapy administration was analyzed by means of synoptical plots. The data provided in the plots were rather anecdotal, especially since the time point of administration of the medication prior to the assay did not vary a lot. They were therefore not considered sufficiently convincing to allow the conclusion that the assay is independent of the administered standard treatments. However, a major effect of ammonia scavenging drugs or other medication, arginine, citrulline and other amino acids on the test results may not be expected given that these drugs do not interfere with the intrinsic capacity of the urea cycle.

2.4.4. Conclusions on clinical pharmacology

Overall, the results of both PD studies CCD07 and CCD09 were not considered adequate to support the claim that the 13C-ureagenesis assay provided a reliable quantitative tool to follow changes in ureagenesis capacity and that it can monitor the effect of curative therapies in UCD; the CAT maintained this as a major objection.

The CHMP endorse the CAT assessment regarding the conclusions on the Clinical pharmacology as described above.

2.5. Clinical efficacy

2.5.1. Dose response study

No clinical dose-response studies were performed. The HHLivC dose used in clinical trials was defined based on the assumptions that 1) as many cells as possible should be administered to achieve a maximal compensation of the metabolic defect and 2) the maximal dose administrable is limited for safety reasons. An increasing number of administered cells also increases the likelihood of deleterious events like portal vein thrombosis and/or shunting of cells into the systemic circulation and lungs.

The chosen dose was based on a preclinical experiment that indicated that an allogeneic liver cell dose corresponding to 5% of normal liver cell mass was sufficient to correct a metabolic defect in a mouse model. The proposed dose also took into account the changing relationship of bodyweight to liver cell mass. These calculations were done based on estimates of liver volume, hepatocyte volume and the changes of liver volume with increasing age. From these calculations the following doses were derived:

Paediatric patients who weigh:

- ≤10 kg receive 0.3 x 10^9 viable liver cells per kg of body weight
- >10 to 15 kg receive 3.0 x 10^9 viable cells (i.e. a fixed dosage non-adjusted to body weight to avoid underdosing)
- >15 kg receive 0.2 x 10^9 viable liver cells per kg of body weight

2.5.2. Main studies

Two pivotal clinical studies with comparable design (CCD02 in Germany and CCD05 in US/Canada) were performed to assess the safety and efficacy of HHLivC in children with UCDs. Interim clinical study reports (iCSRs) were prepared on the basis of the data available at the time of data lock at submission of the application.

A retrospective database study (CCD10) was set up to capture the clinical course of a paediatric subpopulation suffering from UCD with comparable clinical severity as the population in studies CCD02/CCD05, but treated with standard of care only. Data from this non-interventional study were intended to serve as comparison for the clinical studies CCD02 and CCD05.

CCD02: Phase II Open, prospective, uncontrolled, multicentre, with multiple applications of liver cell solutions.

Methods

Study Participants

The participants were treated in only 1 German hospital out of the 5 German centers initiated, including only 2 recruiting centres.

Diagnosis and main criteria for inclusion and exclusion CCD02:

Main Criteria for inclusion

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

- Carbamoylphosphate synthetase 1 deficiency [CPS1D], or
- Ornithine transcarbamylase deficiency [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

Main Criteria for exclusion :
-Structural liver disease (cirrhosis, portal hypertension), or veno-occlusive diseases
-Portal vein thrombosis
-Body Weight ≤3.5 kg
-Any contraindication for immunosuppression
-Required valproate therapy
-Severe coagulopathy or thrombocytopenia.

***Treatments* CCD02**

Liver cells
The “Human Heterologous Liver Cells (for infusion)” is a suspension of liver cells which is prepared from non-transplantable human donor organs. It was manufactured on behalf of Cytonet GmbH & Co. KG.

Production and release of the HHLivC cryopreserved liver cell suspensions was performed in compliance with the current US and EU-GMP and US Good Tissue Practice (GTP) regulations.

Medical device/catheter

The day before Heparesc infusion, a Hickman/Broviac catheter has to be placed surgically into the portal vein via branches of the inferior or superior mesenteric vein. Under general anaesthesia, an upper median laparatomy has to be performed. The middle colic vein is prepared after opening of the omentum majus. The distal end of the vein is ligated, the proximal end incised and the catheter, which has to be tunnelled and has a subcutaneous cuff (preferably on the left side of the abdomen to facilitate liver ultrasound during the cell applications), will be introduced. After the proper position of the catheter (if possible in the portal vein stem to facilitate measurement of portal venous pressure) is confirmed by means of contrast radiography, the catheter is secured at the entry side using a suture that is rapidly absorbable. If the middle colic vein is not suitable for catheter insertion, the large bowel has to be exposed and 5 cm proximal to the left flexure the inferior mesenteric vein has to be prepared.

It has to be confirmed that the Ductus Venosus Arantii is not patent (e.g. using Doppler ultrasonography). In case patency has been observed, the ductus has to be closed surgically during the catheter placement procedure. The abdominal cavity has to be closed and the catheter should be saved with a stay suture.

Due to the general anaesthesia, regular feeding patterns might be disturbed. To avoid catabolism during that period, enteral feeding will be replaced by intravenous glucose supply. For the same reason, oral medication may be replaced by intravenous medication. Ammonia levels will be determined prior to the procedure and regularly until the patient is under the care of the metabolic physician in the children's hospital again.

Antibiotic prophylaxis should be considered according to the site's standard of care (e.g. from time of catheter placement into the portal vein until the catheter is removed).

At an appropriate time after finishing the application of the cells and at the end of the therapy, the catheter will be removed. In most cases, this can be done without relaparotomy after resorption of the securing suture at the vessel wall. However, surgical removal might be necessary. A worksheet for a thorough documentation of times and steps of preparation and procedures during placement of the portal vein catheter and of after-care may be used.

Infusion

An intraportal infusion is considered to deliver into the recipient's liver in humans. Administration of the investigational product took place the day after the placement of the application catheter.

A continuous infusion of a saline solution containing up to 5 IU of heparin/ml was used at a flow rate of 2 to 5 ml/h to maintain patency of the application catheter between HHLivC infusions. This rinsing solution was connected to the application catheter by a 3-way valve that was used for the infusion of the liver cells. A pressure transducer was attached to this line to record PVP.

If possible, the patient received liver cells of AB0 identical blood type.

Doses

The total dose of viable liver cells was up to 0.3 $x10^9$ (300 million) cells per kg body weight divided into individual doses of up to 0.05 x 10^9 (50 million) liver cells per kg body weight. The concentration of the cells in the specific bag was given by the manufacturer. If the cell suspension contained >15 million cells/ml after reconstitution the suspension could be diluted with Composol to a maximum volume of 15 ml/kg body weight. It had been shown that a content of >15 million cells/ml may impair the measurement of portal vein pressure due to increased viscosity in the catheter (experience from the first three study patients).

From the HHLivC specifications and the dosages foreseen in the clinical protocol, a maximum of 15 ml per kg BW was infused per session, thus constituting the daily dosage with one sixth of the total dose according to the body weight of the patient.

Six (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.

Number of cells per individual session (planned)

Body weight	Number of viable cells per individual session	Maximum of total cells infused per individual session (Based on a minimum of 50 % viable cells)	Total Number Viable cell dosage
≤10 kg	0.05 x 10^9 per kilogram of body weight	0.10 x 10^9 per kilogram of body weight	0.3 x 10^9 per kilogram of body weight
>10 to 15 kg	0.5 x 10^9 non adjusted to body weight	1.0 x 10^9 nonadjusted to body weight	3.0 x 10^9 nonadjusted to body weight
>15 kg	0.033 x 10^9 per kilogram of body weight	0.066 x 10^9 per kilogram of body weight	0.2 x 10^9 per kilogram of body weight

Cells reconstitution

For the preparation of the study medication prior to administration, after identification and preparation of the respective patient, the anticipated cryobags with liver cell suspension were consecutively thawed, washed with Composol PS®, and divided into 20 ml syringes or 60 ml syringe that were appropriately labelled. The exact procedures were specified to the investigator.

Monitoring of infusion

During application of the cells, portal vein flow (PVF) has to be monitored constantly by means of Doppler ultrasound. During the application, infusion will be discontinued every time the PVF is decreased to less than 50 % and maybe continued once the PVF has increased.

Measurement of Portal Vein Pressure during HHLivC Infusion

Determination of the portal vein pressure (PVP) via the application catheter has to be done immediately before starting each session. If the portal vein pressure is > 15 mmHg the application cannot be started. The application of the cells is paused every five minutes to measure PVP. If the initial PVP is < 8 mmHg, any increase in PVP up to a physiological limit of 15 mmHg during the application is tolerable. Should the initial PVP be ≥ 8 mm Hg, then a twofold increase of PVP is tolerated with an absolute maximum of 22 mm Hg (150% of the physiological cut-off). Infusion has to be stopped every time the maximum tolerable PVP is reached.

Table 10: Tolerable portal vein pressure (PVP) before and during cell application

Initial PVP	Tolerable increase of PVP / Maximum tolerable level
< 8 mmHg	15 mmHg
8-15 mmHg*	Two-fold increase up to a maximum of 22 mmHg
*Liver cell infusion cannot be started in case of PVP > 15 mmHg	

Monitoring of blood oxygen saturation will be performed before and during each session**.** The value of the oxygen saturation must not fall below 90 % (= limit value). If the oxygen saturation is <90 % before the session the cell infusion cannot be started. If the oxygen saturation falls below 90% during the session the application has to be interrupted. If the oxygen saturation does not increase to ≥90 % within 5 minutes

the cell infusion has to be terminated immediately and the patient has to be closely observed for respiratory complications.

Concomitant medication

All patients received best medical care, liver cell application and immunosuppression (standardized immunosuppressive therapy with tacrolimus or cyclosporine and methylprednisolone or prednisone).

Patients should receive immunosuppression with a calcineurin inhibitor (tacrolimus or cyclosporine) 6 to 12 hours before the first HHLivC infusion and methylprednisolone or prednisone starting at the day of the first LCI at a dosage of 15 mg per m^2 of body surface in two divided doses. After the last application, the dosage is:

week 1	15 mg/m^2	2 divided doses
week 2	12 mg/m^2	1 daily dose
week 3	10 mg/m^2	1 daily dose
from week 4	8 mg/m^2	1 daily dose

Immunosuppression is maintained throughout the study.

The recommended initial oral dose of the CNI is 0.3 mg/kg bodyweight for Tacrolimus and 10 mg/kg bodyweight for Cyclosporin. In this protocol, steroid therapy was initiated with maintenance type doses aiming at reduction of the total immunosuppressive load in newborn children.

For both CNI drugs, the recommended initial oral dose is selected to achieve desired trough levels of the CNI within approximately 10 days. For Tacrolimus, dosage was adapted to daily monitored blood levels throughout the application period to reach trough levels of 5-10 ng/ml (5-15 ng/ml until amendment to protocol version 3.0). If the centre preferred to use cyclosporine A (tacrolimus or cyclosporine by amendment to protocol version 2.4) (Sandimmun®, Roche), oral application was started 6-12 hours prior to the first cell infusion at a dose of 10 mg/kg body weight in two divided doses and was maintained throughout the study. Dosage was adapted to reach trough levels of 180-220 ng/ml up to week 4, 150-200 ng/ml during weeks 5-12, 130-180 ng/ml during the first year (if applicable), and 60-80 ng/ml thereafter (if applicable).

Other treatment

Best medical care was provided to the patient throughout the whole study period. In case metabolic crises occurred or signs of infection became obvious, any procedure taken under the provision of best medical care was permitted.

Detoxification:

-Ammonia scavenging drugs, and other specific drug therapy.

-Extracorporal detoxification (e.g. hemodialysis, hemofiltration).

Duration of monitoring

The active treatment is followed by an observation period of 6 weeks and 8 weeks- listing phase for OLT, total 15 weeks after first infusion.

End of Study: the final visit (FV) of those patients who undergo transplantation is marked by the acceptance of the organ by the surgeon. The study terminates with the Follow-up visit 3 months after OLT. The liver transplantation procedure itself is not part of the study.

In patients who do not receive a liver transplant, the clinical phase of the study terminates 15 weeks after liver cell application with the final visit (Day 105: about 3 months post LCI). They will be followed up for 24 months. The Follow-up-Visits (FUV1 to FUV5) will be conducted 3, 6, 12, 18 and 24 months after the Final Visit.

***Objectives* CCD02**

Primary

- 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.

Secondary

- Efficacy : Changes in 13C urea formation at 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 13C-ureagenesis tests were determined after infusion of HHLivC.

***Outcomes/endpoints* CCD02**

Safety variables:

- Vital signs

- Laboratory Parameters to monitor the safety of the procedures and immunosuppression

- Adverse events

Efficacy variables:

- Changes in ^{13}C urea formation* from baseline to months 2 and 4 (or earlier, if OLT is performed during listing period) and if available up to month 24 (FUV 5) after the Final Visit in case further ^{13}C-ureagenesis tests were determined after infusion of HHLivC.

- Change in the enzyme activity of samples from the explanted liver taken after orthotopic liver transplantation 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 orthotopic liver transplantation 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: 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

- Time to death and Survival 6 months after liver cell infusion

* The ^{13}C-ureagenesis assay was introduced during the clinical trial and was not applied to the 6 first patients.

***Sample size* CCD02**

13 evaluable patients (not more than 20 patients in total) were planned to be recruited at the study centres.

No formal sample size calculation was performed. The number was chosen on a pragmatic basis.

***Randomisation* CCD02**

Not applicable in this open study. Due to the rare availability of patients and since no change of enzymatic activity is expected without OLT or liver cell infusion, no control group was investigated.

***Blinding (masking)* CCD02**

The study was not independent evaluator blinded.

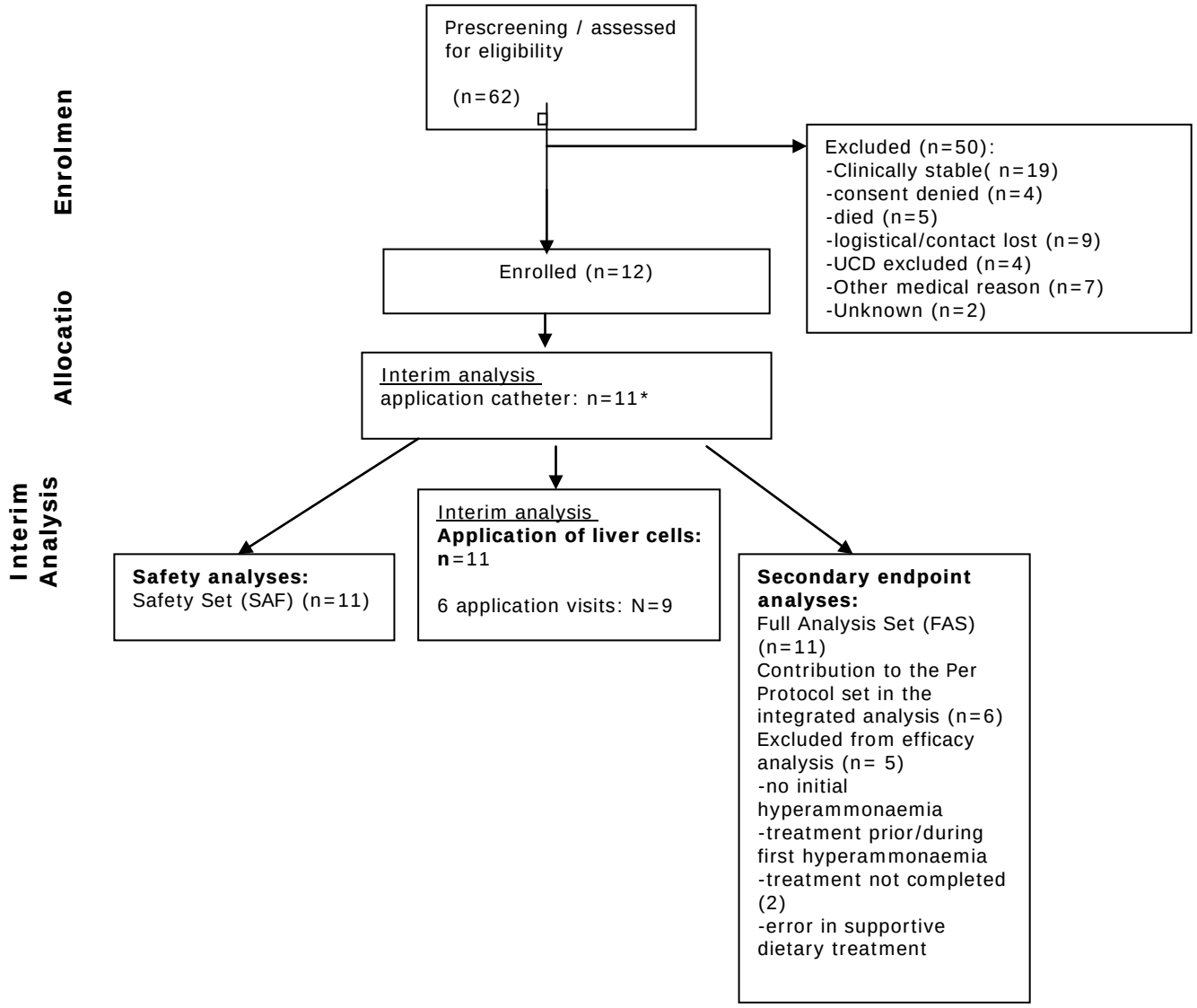
***Statistical methods* CCD02**

According to the amended study protocol, only descriptive statistical methods were applied.

Results

Participant flow in study CCD02

Study participant flow for patient data in CCD02 (at initial submission) was as follows:



With the Responses to Day 120 LOQ, additional patient data were added, bringing the total number of patients treated with 6 HHLC infusions in study CCD02 to 12 patients.

For efficacy analysis the selected population is described in the integrated analysis including both study population from CCD02 and CCD05.

Recruitment

The first patient was recruited in the study on 21 August 2009. A majority of prescreened patients was not enrolled (see table above).

Conduct of the study

The study protocol was amended on 9 occasions. Some assays have been developed during the study. Some patients have not benefited from these tests used for assessing the efficacy variable which generates a disparity in the analysis of the results. Inclusion criteria have been modified (inclusion of older patients, modified as in the PIP), which can lead to heterogeneity of the population and complicate assessment.

The number of deviations from the protocol was rather extensive. Regarding missing or delayed deviations for laboratory evaluations this is understandable considering the paediatric population, but not ideal.

Baseline data

Patients had a confirmed mutation in the affected gene. Most patients had OTCD; one patient with CPS1D was included; the remaining patients had ASSD. The majority were diagnosed upon first manifestation of symptoms and the majority became symptomatic shortly after birth. There were two patients who were diagnosed either prenatally as they had an affected sibling or immediately postnatal as the mother was a known carrier of a UCD mutation.

Numbers analysed

Twelve patients were included in this study, 10 of which received the 6 planned infusions, 2 received only one or a partial infusion.

Outcomes and estimation

Endpoints are presented in table 1 (below) and study results are discussed under the section Analysis performed across trials.

Ancillary analyses

N/A

Study CCD05 : Open, prospective, historic-controlled, multicenter design with multiple applications of Human Heterologous Liver Cells for infusion.

Methods CCD05

14 sites throughout the United States (n=13) and Canada (n=1) have been initiated into the study. To date, 5 centers have enrolled subjects. (4 USA, 1 Canada)

Study Participants CCD05

Main inclusion Criteria

1. Males or females whose gestational corrected age (calculated from term delivery or 37 weeks of gestation) on the day of enrolment was 1 day up to 5 years of age.

2. Complete OTCD, CPS1D, or ASSD with neonatal-onset type (OTCD, CPS1D: clinical presentation with plasma ammonia > 500 µmol/L within the first week of life; ASSD: clinical presentation with plasma ammonia > 500 µmol/L within the first 4 weeks of life) or prospectively diagnosed relative of a subject with the same confirmed diagnosis of complete OTCD/CPS1D/ASSD. Early information about the disease (eg by prenatal diagnosis or newborn-screening) may enable early treatment, which can prevent rising of ammonia to levels >500 µmol/L despite neonatal onset type (complete deficiency). However, in such cases a subject may be eligible after thorough check of the data available (eg DNA analysis) by the investigator.

Further biochemical parameters and DNA analyses were used to confirm diagnosis prior to or after inclusion in the protocol according to the following diagnostic criteria:

OTCD	• Identification of pathogenic mutation and/or • Pedigree analysis and/or <20% of control OTC activity in liver and/or • Elevated urinary orotate (>20 µM/mM) after allopurinol challenge test
CPS1D	• Decreased (<20% of control) CPS-1 enzyme activity in liver and/or • Identified pathogenic mutation*
ASSD	• >10 fold elevation of citrulline in plasma and/or decreased ASS enzyme activity in cultured skin fibroblasts or other appropriate tissue and/or identified pathogenic mutation

*A mutation analysis to exclude NAGSD is required to differentiate from a suspected CPS1D diagnosis. DNA analysis for pathogenic mutation of CPS-1 is planned after enrolment.

3. Plasma ammonia level ≤250 µmol/L at the time of enrolment.

Exclusion Criteria

Subjects were excluded from this study if s/he met any of the following criteria:
-Weight ≤3.5 kg.
-Structural liver disease (e.g., cirrhosis, portal hypertension) or venoocclusive diseases.
-Portal vein thrombosis.
-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.
-Prothrombin time (PT) or partial thromboplastin time (PTT) (or activated partial thromboplastin time [aPTT]) of >1.5 times the upper limit of normal OR platelet count < 50,000 mm^3
-Required valproate therapy.

Treatments CCD05

Subjects received standard-of-care therapy plus an HHLivC infusion and immunosuppression (standardized using calcineurin inhibitors and methylprednisolone or oral equivalent).

The planned viable cell dosage and the method of administration was the same as for study CCD02.

Duration

The study consisted of an application phase (1 week), observation phase (through 6 months or until OLT), Final Visit (FV, 6 months after first HHLivC infusion or earlier in case of premature discontinuation), and a follow-up phase (until 24 months after the first HHLivC infusion or until 3 months after OLT).

Prior and Concomitant Medications and Non-drug Therapies

Antibiotic prophylaxis was considered according to the sites standard of care, (e.g. from time of catheter placement until the catheter was removed).

With respect to cytomegalovirus (CMV), Epstein-Barr virus (EBV), toxoplasmosis, and fungal infections, screening and prophylaxis/ treatment according to local standard for immunocompromized subjects was carried out in the study subjects. If the subject was an EBV-negative recipient, the subject was treated according to the site's standard of care for prophylaxis of EBV infection to minimize the risk of post-transplant lymphoproliferative disorders. Quantitative polymerase chain reaction (PCR) for EBV was regularly assessed to detect an increase of the viral load and initiate appropriate treatment according to the site's standard of care, if necessary.

Objectives CCD05

Primary
Safety and efficacy of multiple HHLivC infusions, in children with OTCD, CPS1D, or ASSD.

Secondary
Safety and efficacy of multiple HHLivC infusions

Outcomes/endpoints CCD05

Primary efficacy variable
The Change in ^{13}C urea formation from Baseline to 2 and 4 months (or earlier, if orthotopic liver transplant [OLT] is performed prior to V19 (17 weeks), after first HHLivC infusion. The plasma concentration of ^{13}C urea at any time point was measured. The following parameters were presented at Baseline and after HHLivC therapy (per visit) and per individual subject:

- the peak level of ^{13}C urea concentration in plasma [C_{max}]
- the time when the peak level of ^{13}C urea concentration in plasma is reached [T_{max}]
- AUC $_{(0\text{-}120min)}$
- AUC $_{(0\text{-latest time point})}$

Secondary efficacy variable
- Number, duration and severity of metabolic crises (maximum ammonia concentration, duration of coma).
- Growth and protein intake.
- Use of ammonia scavenging drugs.
- If an OLT is received, increase in the respective enzyme activity in samples from the explanted liver taken after OLT compared with the enzyme activity in the liver biopsy taken prior to the first HHLivC infusion (only OTCD and CPS1D).
- If an OLT is received, detection of donor cell material in samples from the explanted liver taken after OLT will be investigated and samples will be compared with the liver biopsy taken prior to the HHLivC infusion.
- Laboratory parameters: plasma ammonia, glutamine, urea, and in OTCD : urine orotic acid concentration
- Survival at 6 months after first HHLivC infusion.

Sample size CCD05

Data from 8 subjects were included in the analyses submitted and focused mainly on safety parameters via use of descriptive statistics. Initially 20 treatment subjects plus 20 controls had been planned.

Randomisation

Not applicable in this study

Blinding (masking)

Not applicable in this study

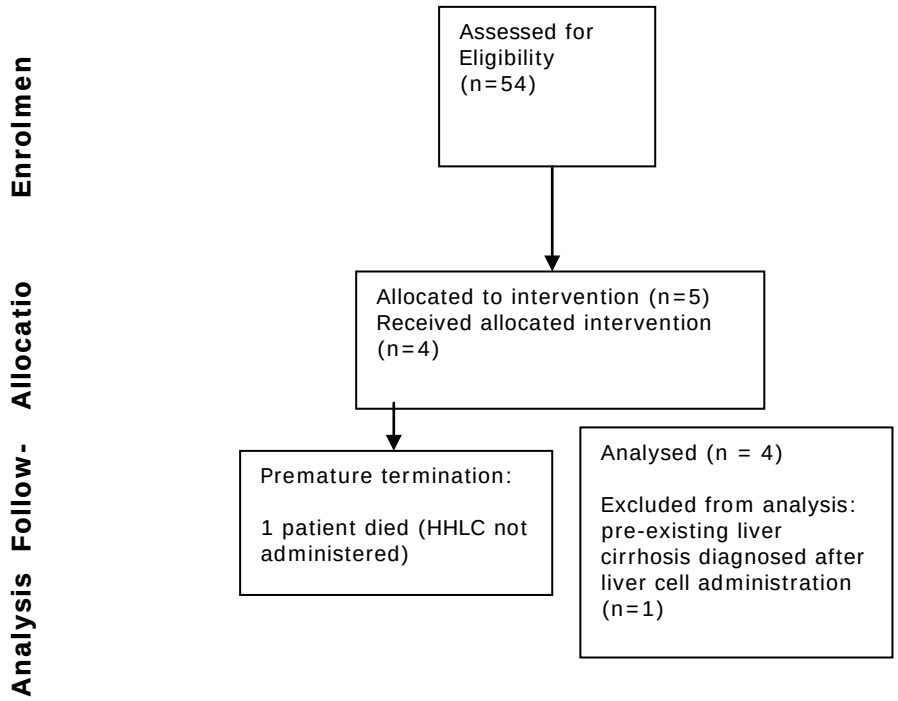
Statistical methods CCD05

Only descriptive statistical methods were applied for this interim analysis.

Results

Participant flow

Study participant flow for patient data in CCD05 (at initial submission) was as follows:



With the Responses to Day 120 LOQ, additional patient data were added, bringing the total number of patients allocated to intervention to 8, and patients receiving HHLC infusions in study CCD05 to 7 patients.

For efficacy analysis the selected population is described in the integrated analysis including both study population from CCD02 and CCD05.

Recruitment

Conduct of the study

Similar to study CCD02 there were a number of deviations related to laboratory assessments and visit schedules. These delays and omissions may be explained by the challenges posed by the study population, however, there may also be element of lacking organisation and oversight at the study centers. These observations emphasise the fact that only appropriately trained personal in appropriately equipped centers can perform this treatment.

There were 3 protocol amendments; these consisted of clarifications and adaptation of the protocol to account for local practices.

Baseline data

In study CCD05, most patients had ASSD, only one patient with OTCD was included. All but one patient were either diagnosed prenatally or in the neonatal period.

Numbers analysed

Data from eight patients were submitted, seven of which received the 6 planned infusions of HHLivC. One patient did not receive the treatment because of increased portal vein pressure.

Outcomes and estimation

Endpoints are presented in table 1 (below) and study results are discussed under the section Analysis performed across trials.

Ancillary analyses

N/A

Integrated analysis of studies CCD02/CCD05 and CCD10 (historical controls)

An integrated analysis has been performed by the applicant based on data from the exploratory studies CCD02/CCD05 and the historical control study CCD10.

METHODS integrated analysis

Study participants (integrated analysis)

Inclusion criteria:

The participation in one of the clinical studies CCD02 and CCD05, or the participation in the database study CCD10. Target population is patients with documented neonatal onset disease of UCD (OTCD, ASSD, CPS1D).

Three analysis sets were identified:

-The **Safety Set** comprised all patients who received at least an attempt for placing an application catheter for liver cell suspension (CCD02, CCD05) and all patients documented in CCD10. This set was used for all safety analysis.

-The **FAS (full analysis set):** all patients who respected the inclusion and exclusion criteria for the integrated analysis:

Exclusion criteria from the FAS

- No initial ammonia value >500 µmol/l and no documented prenatal diagnosis or documented family history and immediate treatment
- Diagnosis not provided
- Erroneous LCT (i.e. LCT in CCD10 or no LCT in CCD02 / CCD05).

-The PP (treated patient) / RC (relevant control) population (RC set called FAS_{ex} in CCD10 study). Exclusion criteria from the PP/RC set:

- Death in the first hyperammonemic crisis (only CCD10)
- No unstable metabolism (mild disease) as defined by not having had any ammonia levels ≥150 µmol/l within at least 6 months after the end of the initial hyperammonemic crisis (only CCD10)
- LCT not completed (only CCD02 / CCD05)
- Pre-existing liver cirrhosis at LCT and PVP too high
- Start of LCT before and completion of LCT during the first hyperammonemic event (only CCD02 / CCD05)
- Erroneous administration of toxic protein dose.

Treatments (integrated analysis)

For details, refer to description under the section on study CCD02 and CCD05 respectively

- Heparesc and Immunosuppressive treatment
- Standard treatment: Ammonia Scavenging Drugs

Objectives (integrated analysis)

The primary objective of the integrated analysis was to assess the efficacy of multiple applications of liver cell suspension in children with UCDs in the overall data set of the CCD02 and CCD05 studies in comparison with corresponding data derived from the retrospective documentation in study CCD10.

Efficacy Parameters in Studies CCD02, CCD05, and CCD10

CCD02	CCD05	CCD10
Primary endpoints: • 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	**Primary endpoint:** Changes in ^{13}C-urea formation from baseline to 2 and 4 months (or earlier if OLT is performed prior to month 4 [V19]) after first HHLivC infusion	**Primary endpoints:** • Hyperammonemic events • Events of increased glutamine (≥1000 µmol/l) • Severe metabolic crises • Survival
Secondary efficacy endpoints:		
Changes in ^{13}C-urea formation from baseline to 2 and 4 months after first HHLivC infusion (or earlier if OLT is performed) and, if available, up to 24 months after the final visit*	See primary endpoint	NA
Number, duration and severity of metabolic crises (maximum ammonia concentration, duration of coma)	Number, duration and severity of metabolic crises (maximum ammonia concentration, duration of coma)	Number, duration and severity of metabolic crises/coma/ hyperammonaemic events (see primary endpoints)
Change in enzyme activity in explanted liver in case of OLT compared to baseline enzyme activity.	Change in enzyme activity in explanted liver in case of OLT compared to baseline enzyme activity (only OTCD and CPS1D).	NA
Detection of donor cell material in samples from the explanted liver taken after OLT compared with the liver biopsy taken prior to HHLivC infusion	Detection of donor cell material in samples from the explanted liver taken after OLT compared with the liver biopsy taken prior to HHLivC infusion.	NA
Laboratory parameters: ammonia, amino acids and urea in plasma and orotic acid in urine (except CPS1D)	Laboratory parameters: ammonia, amino acids and urea in plasma and orotic acid in urine (OTCD only)	Laboratory parameters: ammonia, glutamine (see primary endpoints)
Use of ammonia scavenging drugs Growth and protein intake Nutritional Status Survival at 6 months after LCI	Use of ammonia scavenging drugs Growth and protein intake Nutritional Status Survival at 6 months after LCI	Use of ammonia scavenging drugs Growth and protein intake Survival (see primary endpoints)

* Introduced while study was ongoing
CPS1D = Carbamoylphosphate synthetase type 1 deficiency, HHLivC = human heterologous liver cells, NA = not applicable, OLT = orthotopic liver transplantation, OTCD = Ornithine transcarbamylase deficiency
Source: CCD02, CCD05, CCD10 CSRs.

Outcomes/endpoints (integrated analysis)

Efficacy

Primary endpoints

-Time to death, survival

-Time to severe hyperammonemic events, defined by plasma ammonia ≥500 µmol/l (including death)

-Time to at least moderate hyperammonemic events, defined by plasma ammonia ≥250 µmol/l (including death)

-Time to at least mild hyperammonemic events, defined by plasma ammonia ≥150 µmol/l (including death)

-Incidence of hyperammonemic events (including death) using the cutoffs 150, 250, and 500 µmol/l

Secondary endpoints

-Events of increased glutamine, defined by glutamine levels ≥1000 µmol/l

-Hyperammonemic events which caused hospitalization, coma, i.v. ASDs, dialysis

-Arginine, citrulline, and urinary orotic acid

-Need of oral ASDs.

Safety

- Incidence of successful catheter placement
- Adverse events during liver cell infusion and before, during, and after analysis period
- Safety laboratory tests during liver cell infusion and during analysis period
- Vital signs during liver cell infusion and during analysis period
- Physical examination during liver cell infusion and during analysis period
- Growth and nutritional status.

Statistical methods (integrated analysis)

Various statistical tests (t-test, F test, U test, Kruskal-Wallis test to compare location parameter from independent groups, $\chi 2$ test to compare frequency distributions, logrank test / generalized (Gehan-Breslow-)Wilcoxon test to assess the time event data etc.) were used to compare the data from studies CCD02/CCD05 to the historical controls from CCD10.

All tests were decided on the significance level of $\alpha = 0.05$.

Summary of main efficacy results

The applicant performed two single arm trials CCD02 and CCD05 in patients with UCD to support this marketing authorisation; the studies were still ongoing at time of submission of the application for marketing authorisation. Both studies were open, prospective, uncontrolled studies. The study population were neonates and infants up to the age of ≤3 months OR children aged >3 months up to less than 6 years of age with unstable metabolism, diagnosed with 1 of the following UCDs: CPS1D, OTCD and ASSD. The treatment consisted of 6 administrations of HHLivC on 6 consecutive days via an implanted catheter into the portal vein. All patients received best standard of care as background therapy. Concomitant therapy with immunosuppression was necessary to enable engraftment of allogeneic cells.

An updated integrated analysis (with the addition of four patients) was submitted in response to the d120 LoQ and in response to the d180 LoOI. An updated study report has not been provided.

A total of 12 patients was enrolled in study CCD02. A total of 8 patients enrolled in study CCD05. All patients included in both trials had confirmed UCD.

The analysis of the efficacy with respect to clinical endpoints (hyperammonaemia, survival, development) within these trials was hampered because of basic methodological reasons, by the population and disease characteristics (rarity, different mutations, variability of clinical course, different phenotypes) and therefore regarded as not conclusive. Looking at data regarding hyperammonaemia it appeared that the distribution of hyperammonaemic events had no discernible pattern and severe hyperammonaemic events were not prevented in treated patients.

Because of the difficulty in interpreting efficacy data in the single arm trials the applicant conducted an integrated analysis using available clinical data in comparison to a historic control derived from the CCD10 data collection which is summarised in table 1 below.

The data on the historical control cohort CCD10 were collected in eight European centres and encompassed 63 patients. Data on hyperammonaemic events, laboratory values, adverse events, death, liver transplantation and their respective time points in relation to date of diagnosis and date of birth were collected.

The investigation of urea generation capacity with the 13C ureagenesis assay is supportive information that was collected within these two trials. For study CCD02 it was introduced in the course of the trial, for CCD05 it was considered the primary endpoint.

In CCD02 6 patients were studied with this test, in CCD05 all 8 patients were studied. Pre-treatment data are available in 11 patients and post-treatment data for up to 24 months in 12 patients. Three patients of 12 with post-treatment data have no pre-treatment data (analytic failure, not done). Of 9 patients with both pre- and post-treatment data, 6 showed changes in the ureagenesis assay that taken at face value indicated some change in metabolic activity as reflected in an increase of the AUC_{0-120}. The observed changes reached statistical significance. However, when comparing ureagenesis data generated in different patient populations with distinguishable severity of clinical disease to the changes observed in the treated patients these changes are only marginal and clinical relevance can hardly beattributed.

The provided data on assay validation and assay performance (as discussed in section 2.4.3) in the opinion of the CAT also do not contribute to concluding on clinical relevance.

Analysis performed across trials (pooled analyses and meta-analysis)

The following table summarises the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 11. Summary of Efficacy for integrated analysis of CCD02, CCD05 and CCD10

Title: Combined Analysis for all patients recruited in the clinical trials CCD02 and CCD05 and patients readout from CCD10 data base as historic controls			
Study identifier	Integrated analysis		
Design	Integrated analysis of patients included in the phase II studies CCD02, CCD05 compared to the retrospectively collected population described in study CCD10		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		**CCD02 : 21 Aug-2009 to 11 Jan 2013** **CCD05 : 13 Dec 2010 to 26 Apr 2013** **CCD10 : Feb 2001 to Feb 2013 (range of documentation periods)** not applicable Studies CCD02 and CCD05 ongoing
Hypothesis	Exploratory: comparison of efficacy of HHLivC in the overall data set		
Treatment groups	CCD02/05		6 HHLivC administrations over 6 days 20 patients overall
	CCD10		Historical cohort 63 patients overall
Endpoints and definitions	Primary efficacy variable	Hyperammonaemic events	Hyperammonaemic events (HE), defined by ammonia levels ≥ 500 µmol/L (severe), ≥ 250 µmol/L (moderate), ≥ 150 µmol/L (mild), analysis of incidence and time to event or death
	Primary efficacy variable		Survival

	Secondary efficacy variable	Glutamine elevation	Events of increased glutamine, defined by glutamine levels ≥ 1000 µmol/L
Database lock	Unknown		

Results and Analysis

Analysis description	**Primary Analysis*** *: 2nd analysis, as submitted in response to D120 LOQ		
Analysis population and time point description	Per protocol / relevant control: selected subpopulation of the FAS		
Descriptive statistics and estimate variability	Treatment group	CCD02/05	CCD10
	Number of subject	12	19
	Individual maximum level of ammonia	243 µmol/l	438 µmol/l
	Median number of HE in analysis period (including deaths) U-test p = 0.21	1.5	3.0
	Deaths Chi2 test p = 0.089	0	4
	Individual maximum event <150 µmol/l 150-249 µmol/l 250-499 µmol/l >499 µmol/l	 2 5 4 1	 - 2 6 7
	Number of severe events >500 µmol/l	1	11
Notes			

Analysis description	**Integrated analysis of data from open, single arm exploratory phase trials. The efficacy analysis has no single prespecified endpoint. The control population "relevant control" is a selected set of patients from the historical cohortCCD10 (n=63) as presented by the Applicant. Main reason for exclusion from the "relevant control" set are no initial severe hyperammonaemia, death in the first hyperammonaemic episode, and mild disease within the first 6 months after diagnosis. The data in this table are a selection from multiple comparisons based on the perceived importance of the endpoint.**

In the applicant's responses to day 180 List of Outstanding Issues, considering major objection raised by the rapporteurs the selected population for efficacy analyses were further modified (treated and control group). An expert panel managed by the applicant determined which patients of the treated population and which patients of the control population should be included in the analysis. After assessment of new criteria established, 14 patients were allocated to treated group with Heparesc and 27 patients allocated to control group. The aim of this exercise was to obtain a better matched control population based on inclusion/exclusion of the clinical trial CCD02/CCD05 (see Efficacy data and additional analyses).

Supportive studies

There are no supportive clinical studies (other than the 13C ureagenesis study described above under section 2.4.3).

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The applicant started the development with study CCD02 which was also the first study with HHLivC in this population. This study was an open label study including patients with confirmed UCD (CPS1D, OTCD, ASSD) up to the age of five. For patients older than 3 months an unstable metabolism was a further inclusion criterion. While the study population was acceptable in principle, it is likely that patients were included in the trial based on the choice of their treating physician. This bias was considered of critical importance for the evaluation of the data. The direction of this bias was unclear. The applicant presented the view that investigators were reluctant to include stable patients into a trial with a new and experimental treatment therefore concluding that only unstable patients were included. However, also neonates were included, in which case there was no information on the stability or instability of the disease. The applicant acknowledged that a definition of "instability" had not been possible. It is not unlikely that patients were only included if they had no severe neurological sequelae from the previous hyperammonaemic episodes, which could indicate a milder disease phenotype overall.

The study was an exploratory phase I/II study (also reflected in the fact that efficacy endpoints were considered secondary). Treatment consisted of a total of 6 doses of HHLivC, the dose was body weight adjusted and given on 6 consecutive days via a catheter that had previously been placed to allow administration into the portal vein. During administration the pressure in the portal vein was monitored and application speed was adjusted. All patients received the intervention on the background of standard therapy, which is acceptable.

The prevention of hyperammonaemic events was considered as an acceptable surrogate for efficacy, with hyperammonaemia being regarded as the main cause of damage to the CNS. There is no generally accepted upper threshold that would exclude ensuing CNS damage and thus the definition of hyperammonaemic events was somewhat arbitrary. The company classified hyperammonaemic events

by using cut-offs of 150, 250 and 500 µmol/L. Other endpoints were mortality, changes in amino acid levels and the development of the children.

Patients were recruited from many different centres but treated almost exclusively in one centre in Europe. The complexity and the novelty of the intervention possibly explained a number of deviations that were observed in the conduct of the study, therefore an inspection was requested. The inspection at the clinical site and at the sponsors revealed critical and major findings related to study design, conduct and oversight of the study, safety reporting, IMP handling, monitoring of the trial and informed consent handling. It was concluded that the trial did not conform to GCP. However, although the CAT members acknowledged the findings reported by the inspectors, the majority of the CAT members were of the opinion that in the context of the specific disease and the specific intervention an evaluation of the benefit/risk through the data gathered was still feasible and the assessment continued. During the conduct of the CCD02 trial a 13C ureagenesis assay was introduced for the assessment of intra-individual changes in urea generation capacity. There was also an attempt for the evaluation of enzymatic activity in explanted livers at the time of orthotopic liver transplantation (OLT).

The second study CCD05 had a very similar design. The population in this study was restricted though to patients with neonatal onset or prenatal diagnosis with complete enzyme deficiency (OTCD, CPS1D, ASSD). Treatment was identical and background treatment conformed to the local standards. In this trial the 13C ureagenesis assay was the primary endpoint, secondary endpoints related to hyperammonaemia, growth and development, use of ammonia scavenging drugs, detection of enzymatic activity or DNA from donor cells at the time of OLT. Also in this study it was apparent that the intervention and its complexity put some strain on the resources of the trial sites as investigations were not performed in the correct way or at the right time. CAT concluded that the impact of the violation of GCP requirements did not invalidate the acquired data in their totality and the assessment continued.

Pooling of both populations for the integrated analysis was acceptable even though there were differences as regards the inclusion criteria.

The retrospective data collection of historical controls in study CCD10 is considered acceptable and the type of data that were collected are relevant. However, the further definition of the “relevant control” based on the course of the disease and exclusion of the majority of control patients is considered critical.

The statistical analyses can be considered as exploratory overall.

In summary the trial design, the choice of the historical control and the multiple analyses could severely bias the conclusions on efficacy. CAT accepts that a randomised trial, much less a blinded trial is not possible in this specific population. However, effect sizes likely have to be larger to be detectable in an uncontrolled setting.

Efficacy data and additional analyses

The applicant presented several lines of argumentation for the demonstration of efficacy as no conventional efficacy trials were conducted.

The evaluation of clinical efficacy relied on two exploratory trials in comparison to a historic control population. Clinical endpoints of interest were survival, time to hyperammonaemic events and incidence of hyperammonaemic events. The analysis of these endpoints followed a pre-specified plan. However, this plan was presumptively developed with the knowledge of the available data as these were open label trials. According to the applicant the presented analyses for these endpoints showed the following:

- Lower risk of death in the experimental group (per protocol set, 0/12 patients) compared to the historical control (relevant control set, 4/19 patients).

- Longer time to first occurrence of an at least mild, moderate or severe hyperammonaemic event (≥500 µmol/l) representing a lower hazard for an at least mild, moderate or severe hyperammonaemia
- Lower incidence rates of hyperammonaemic events (HHLivC 0.167 HE/30 days, control 0.370 HE/30 days)

The planned and performed analyses had a high risk for bias for the following reasons:

- The inclusion of patients into the experimental trials may have been strongly influenced by factors not reflected in the inclusion criteria, namely individual judgement by the referring physicians that may be driven e.g. by medical, scientific or social considerations.
- On the other hand the conclusions were highly dependent on the definition of the control population. The applicant used a population called "relevant control" that constituted a selected subset of the collected control population. This population excluded patients on the basis of the observed natural clinical course, which by definition would not be available for the experimental arm. The results for the time to event analyses appeared to be driven by patients who had events rather early in the analysis period.
- It was also noted that there were baseline differences in experimental and control population which also introduced an unknown bias.
- It is not expected that patients with the experimental treatment would fundamentally be different early in the course of disease when infused cells may not be fully functional yet. The analysis for hyperammonaemic events using different cut-offs above which events are counted leads to inclusion of the severe events (and deaths) in the analysis of the less severe events. The results for less severe events may therefore be biased by the severe events. An updated analysis seemed to indicate that there was a shift in HE severity from severe to mild when comparing populations. It is agreed that the data seem to support this hypothesis however it is difficult to accept the concept of severity shift for a metabolic disease.

The Applicant provided several updated analyses during the MAA assessment procedure, including more patients and also performing bootstrap analyses in an attempt to better match patients and controls. As an additional attempt to obtain a more similar treated and historical control population the applicant convened an expert panel consisting of the investigators and one additional expert. This expert panel determined which patients of the treated population and which patients of the control population should be included in the analysis. The aim of this exercise was to obtain a better matched control population based on inclusion/exclusion of the clinical trial CCD02/CCD05 and to address the CAT concern that a non-specified criterion "physician preference" and early deaths in the control cohort could bias the results. The final "expert panel treated set" included 14 patients and the "expert panel control set" included 27 patients. The Kaplan-Meier curves and related statistics for 1000 bootstrap samples and three time-to-event endpoints (hyperammonaemic events HE≥150 µmol/l, HE≥250 µmol/l, HE≥500 µmol/l at 6 and 12 months (including death)) were updated, using the "expert panel treated set" and the "expert panel control set". As judged by the distribution of p-values of the bootstrap exercise (which included only 13 patients) there was a trend for an advantage in the control group for hyperammonaemic events HE≥150 µmol/l and an advantage for HHLivC for HE≥250 µmol/l and HE≥500 µmol/l. Nevertheless, regarding the distribution of the one-sided p-value of the logrank test for 1000 bootstrap samples, the percentage of p-value<0.05 from bootstrap was very small, i.e. in the range of 1-2%. These very low percentages of p-value<0.05 reflect the overall low level of evidence to support efficacy of the product. In conclusion, even though the concept of this additional analysis was improved and the chosen approach to define a patient population based on criteria developed by the expert panel was acceptable there was no reasonably convincing evidence of efficacy.

In principle, the ureagenesis assay potentially allows an assessment of enzymatic activity in the urea cycle and comparison before and after therapy. However, sensitivity of the assay is unknown and the extent of change in urea generation that is clinically relevant is unknown. Altogether 12 patients were studied for 13Cureagenesis; 9 of those had data that could be evaluated with intra-patient comparison, and 6 patients showed changes in urea generating capacity that could be interpreted as an increase (in ureagenesis) after treatment. The applicant also addressed this issue at the oral explanation held at CAT. It has however not been conclusively demonstrated that the observed changes had clinical relevance.

Direct analysis of enzymatic activity in explanted livers at the time of orthotopic liver transplantation failed. The attempt to discover administered HHLivC cells by means of other techniques (immunohistochemistry, PCR for wild type DNA, etc.) gave mixed results (see above). The information was not quantitative and in the majority indicated presence of donor DNA.

In summary, the CAT was of the opinion that there was no reasonably convincing demonstration of efficacy and only circumstantial evidence that could indicate that some change in ureagenesis is occurring in association with previous HHLivC administration.

2.5.4. Conclusions on the clinical efficacy

The CAT considers that clinical relevance of the observed changes in 13C ureagenesis assay results has not been demonstrated and there are still uncertainties regarding the variability of the data gathered from this assay.

The CAT remain of the opinion that efficacy has not been demonstrated with reasonable certainty (even in the context of conditional MAA with limited data), and it is possible that the observed trends as regards reduction of hyperammonaemic events are chance findings based on a comparison to historical control with too many biases.

Overall there were many methodological issues in the studies conducted and a lack of consistency in the data presented. Data were analysed post-hoc, and many sources of bias were present. In view of the results presented a strong endpoint was missing, and correlation between the different endpoints used was not demonstrated.

The CHMP endorse the CAT conclusion on clinical efficacy as described above.

2.6. Clinical safety

Patient exposure

Overall 19 patients received at least one dose of HHLivC within the clinical trials, 17 patients received the planned 6 doses, 2 did not receive the intended dose. The administered dose of viable cells was 0.180 $x10^9$ to 3.179 $x10^9$ with a mean dose of 1.517 $x10^9$. Thus the experience with patients exposed to HHLivC was limited.

Adverse events

Investigating safety in a complex intervention in a small population with a severe disease is a challenge, thus any conclusions on safety have a great degree of uncertainty. A comparison of safety data of the treated patients with historical control is also problematic. It is expected that there is underreporting for the historical control thus disfavouring the experimental arm.

Any complication from surgery or immunosuppression is regarded to be associated with the intervention and thus relevant for safety. The majority of adverse events were either age-related or related to the

underlying disease and its complications. There were no obvious imbalances other than those caused by the surgical interventions.

Serious adverse event / deaths / other significant events

MedDRA SOC		HHLivC			Control			Fre-quency
		N	(%)	E	N	(%)	E	
Number of patients		20			63			
Infec	Infections and infestations	12	(60.0)	24	28	(44.4)	74	40
Metab	Metabolism and nutrition disorders	15	(75.0)	32	8	(12.7)	13	23
Genrl	General disorders and administration site conditions	7	(35.0)	15	12	(19.0)	16	19
Gastr	Gastrointestinal disorders	7	(35.0)	13	10	(15.9)	15	17
Nerv	Nervous system disorders	4	(20.0)	5	13	(20.6)	23	17
Resp	Respiratory, thoracic and mediastinal disorders	–		–	11	(17.5)	19	11
Vasc	Vascular disorders	2	(10.0)	3	9	(14.3)	13	11
Blood	Blood and lymphatic system disorders	1	(5.0)	1	8	(12.7)	13	9
Inj&P	Injury, poisoning and procedural complications	5	(25.0)	5	3	(4.8)	5	8
Card	Cardiac disorders	–		–	7	(11.1)	11	7
Hepat	Hepatobiliary disorders	2	(10.0)	4	4	(6.3)	6	6
Renal	Renal and urinary disorders	–		–	4	(6.3)	5	4
Cong	Congenital, familial and genetic disorders	3	(15.0)	3	–		–	3
Inv	Investigations	2	(10.0)	2	1	(1.6)	1	3
Surg	Surgical and medical procedures	–		–	3	(4.8)	7	3
Musc	Musculoskeletal and connective tissue disorders	1	(5.0)	1	–		–	1
Skin	Skin and subcutaneous tissue disorders	1	(5.0)	1	–		–	1

Reference: Table 12.204, List 12.3.2.1
N = Number of patients; % = Percentage of patients; E = Number of events

Table: Serious adverse events in CCD02/CCD05 and CCD10 with onset during the analysis period (before OLT, if applicable; CCD10: truncated after 550 Days; at least 20% of patients at the PT level). Safety set

There are no obvious balances caused by the HHLivC intervention as regards AE other than those caused by surgery for catheter implantation. Hyperammonaemia was not collected as an AE in the historical control, thus this imbalance is artificial.

One patient died 83 days after therapy with HHLivC after experiencing a hyperammonaemic crisis. This death is not considered related to HHLivC. Two patients died from complications of OLT, these deaths are also not considered related to HHLivC.

Laboratory findings

Laboratory results were heavily confounded by the necessary concomitant therapies especially immunosuppressive therapies. A transient elevation of liver enzymes was observerd after administration of HHLivC. The cause was speculated to be a combination of release from damaged HHLivC and from the recipient liver as a consequence of perfusion alterations.

Safety in special populations

Not applicable.

Immunological events

Data on allo-sensitisation after exposure to HHLivC were lacking and would need to be generated because of possible long-term implications for the success of OLT.

Safety related to drug-drug interactions and other interactions

The adverse events from immunosuppressive treatment are well described. In the context of the underlying disease and based on the limited sample size it was not possible to detect a specific concern related to HHLivC. It is unlikely that HHLivC interacts with other medicines.

Discontinuation due to adverse events

Two patients discontinued because of either failure in portal vein catheter application or catheter dislocation.

Post marketing experience

Not applicable.

2.6.1. Discussion on clinical safety

Treatment with HHLivC consists of a combination of interventions: the placement of the portal vein catheter within a surgical procedure, administration of cells and concomitant immunosuppressive therapy. The safety of these interventions has to be distinguished from the standard of care which involves dietary measures, ammonia scavenging drugs, dialysis in case of severe hyperammonaemia and the necessary medical interventions e.g. in case of coma.

Based on the limited patient exposure and the lack of a concomitant control it is extremely difficult and rather unlikely to detect any differences in the safety profile. All the known risks for surgery, general anaesthesia and systemic immune suppression have to be taken into account. While it may seem reassuring that no patient died as result of surgical intervention for catheter implantation or because of catheter complications - so far one mild thrombosis of the portal vein was observed - risk of thrombosis is certainly present with the infusion of a considerable amount of dead/dying cells into the portal vein system. Furthermore each intervention in this vulnerable population is able to trigger decompensation. In some patients complications around the time of surgery/HHLivC administration led to transient deterioration of ammonia metabolism.

During the GCP inspection critical findings with an impact on the collection and evaluation of safety data were observed. According to the inspectors the inspected trial site and the sponsor site were not conforming to GCP. Although the CAT members acknowledged the findings reported by the inspectors, the majority of the CAT members were of the opinion that in the context of the specific disease and the specific intervention an evaluation of the benefit/risk through the data gathered was still feasible. In any case, for the inspection findings corrective and preventive actions were requested by the inspectors, especially with regard to any future clinical trials to be conducted by the sponsor.

2.6.2. Conclusions on the clinical safety

The conclusion on safety has a high degree of uncertainty in view of the very limited amount of data available and findings of the GCP inspection. The major risks pertain to cell infusion and portal vein thrombosis, surgery, anaesthesia, complications of surgery and anaesthesia and immune suppression. Fatal events in relation to the intervention were not observed.

The CHMP endorse the CAT conclusion on clinical safety as described above.

2.7. Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CAT considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

The CAT, having considered the data submitted in the application was of the opinion that due to the concerns identified with this application, the Pharmacovigilance system cannot be agreed at this stage.

2.8. Risk Management Plan

The CAT received the following PRAC Advice on the submitted Risk Management Plan:

The PRAC considered that the risk management plan version 1.2 is acceptable. The PRAC endorsed PRAC Rapporteur assessment report is attached.

The CAT, having considered the data submitted in the application was of the opinion that due to the concerns identified with this application, the risk management plan cannot be agreed at this stage.

The CHMP endorse the CAT advice on the RMP.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Labelling exemptions

A request of translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

The QRD Group has accepted the request of translation exemption of labelling taking into account that Heparesc is an orphan medicinal product (Art 63.1) and will not be handed directly to patients but only to health care professionals.

Acceptance by the QRD group is subject to certain conditions and provided that the applicant takes into account previous comments raised on the PI and mock-ups submitted.

The labelling subject to translation exemption as per the QRD Group decision above will however be translated in all languages in the Annexes published with the EPAR on EMA website, but the printed materials will only be translated in the language(s) as agreed by the QRD Group.

3. Benefit-Risk Balance

Benefits

Beneficial effects

The therapy with HHLivC is considered as a partial correction of the urea cycle defect and is intended as a bridging therapy until orthotopic liver transplantation can be performed. Thus the medicinal product does not imply a complete nor permanent cure of the disease.

The analysis for hyperammonaemic events (HE) as provided by the applicant at the time of submission (based on interim data of 16 patients enrolled) showed that patients in the per protocol treatment group (9 patients) compared to the "relevant control" population from the historical cohort (which excluded deaths from the first episode and patients with mild disease course) had a lower incidence of severe hyperammonaemic events (median number of all HE 1.0 vs 3.0 ; median number of HE in 30 days of analysis period 0.2 vs. 0.4 ; HE ≥ 500 µmol/L 0/9 vs. 7/19) including death (0/9 vs 4/19).

Further analyses using Kaplan Meier plots showed a prolonged time to hyperammonaemic events.

Various updates on selected population (14 patients in the treated group and 27 patients in the control group) and new analyses such as additional bootstrap analysis (13 Heparesc treated patients selected) were provided during the assessment of the procedure. Even though the concept of this new analysis was improved and the chosen approach to define a patient population based on criteria developed by the expert panel was acceptable there was no reasonably convincing evidence of a beneficial effect.

Investigations of the capacity of the urea cycle to generate urea by means of the ^{13}C ureagenesis assay showed an increase of $AUC_{0\text{-}120}$ after HHLivC administration in 8 of 9 investigated patients based on intra-individual comparison; however the change in post-treatment values was not considered clinically relevant

Persistence of some donor liver cells/ liver cell DNA in the recipient after treatment was shown but this information was not quantitative.

Uncertainty in the knowledge about the beneficial effects

This application was based on 2 small open label studies in comparison to a historic control population. The conclusion on the beneficial effects was based on multiple analyses of data derived from an exploratory setting. Statistical significance testing should therefore also be regarded as exploratory and not as confirmatory. Due to the selection of the study populations there was a high potential for bias, the analysis of the baseline characteristics further supported this.

The selection of patients into the trials CCD02 and CCD05 was highly liable to bias and dependent on knowledge and personal preference of the referring physician and the patients` parents. The inclusion criterion of unstable metabolic control was ill defined and subjective. The direction of this bias is unknown; it may well be that patients with perceived relatively good prognosis and the capability to adhere to all study relevant investigations were preferentially recruited.

Of 20 patients enrolled in the 2 studies only 12 were included in the per protocol population for the combined analysis), which may be an additional source of bias.

Another issue was the exclusion from the control group of patients with mild disease over the course of 6 months after the initial events. This selection had the potential to severely bias the results in favour of the experimental treatment. Early hyperammonaemic events and deaths were formally (by the data

collection rules for the historical control) not part of the first hyperammonaemic event that typically is observed soon after birth of an affected individual. But these events are in reality an extension of the first manifestation of disease. These rules lead to inclusion of patients in the control group in the unstable early phase at a young age. Also, observed differences, driven predominantly by very early events after treatment, would indicate that infused liver cells are immediately functional to a high degree which is not considered highly probable.

The applicant provided a number of analyses to address the above mentioned causes of uncertainty and bias. Most notably a control population that was selected based on input from an expert panel and an analysis using bootstrap methodology and matching for UCD subtype and age to investigate the time to hyperammonaemic events was submitted and also presented at an oral hearing. Using this modified dataset the observed effect as judged by the Kaplan Meier curves for time to mild, moderate or severe events (≥150, ≥250 or ≥500 µmol/l) was either absent or small. Distribution of p-values of the bootstrap analysis was also not supportive for demonstration of efficacy: an "advantage" was seen for the control group for time to HE≥150 µmol/l that was of comparable magnitude to the advantage observed for the treated group with respect to time to HE≥250 µmol/l or HE≥500 µmol/l. The CAT considered these analyses more credible than the initially presented analyses and was of the opinion that no reasonably convincing favourable effect has been demonstrated.

The ^{13}C ureagenesis assay/data presented did not allow to consider this as an established biomarker, because of uncertainties as regards the performance of the assay. Even when disregarding these assay performance issues and taking the results at face value there was no compelling evidence that the observed changes of intra-individual comparison pre/post treatment have clinical relevance.

Detection of liver cell /liver cell DNA in the explanted liver is weakly supportive for the claim that cells persist, quantitative information is lacking and clinical relevance is unknown. Determination of enzymatic activity in explanted livers was not successful and thus does not support concluding on a favourable effect.

Further concern regarding validity or robustness of the data submitted resulted from the findings of the GCP inspection that was carried out at the investigator and the sponsor sites involved in the CCD02 study. The inspection pointed out 5 critical and 4 major findings at the investigator site and 6 critical and 5 major findings at the sponsor site.

Risks

Unfavourable effects

The following adverse events (AE) were observed: anaemia, hypotension, transaminase increase, rash, pain, haemorrhage and infections. In two patients the portal vein catheter dislocated and treatment could not be continued or even started.

Most adverse events were related to the surgery/anaesthesia, portal vein catheter placement and HHLivC administration. Few adverse events were related to the product itself. However, immediate complications of surgery and anaesthesia were observed and have to be taken into consideration for the safety assessment (even if overall treatable and not permanent). A significant proportion of the patients suffered events that could be attributed to the procedure (10 AE in 6 patients with at least possible relationship to study medication, 33 AE in 15 patients with at least possible relationship to catheter placement) in the analysis of short term safety.

Uncertainty in the knowledge about the unfavourable effects

The sample size was extremely limited and only few patients had been exposed (19 patients in CCD02 and 05). In addition the data were confounded by the underlying disease and its complications, and evaluation was made even more difficult by the challenges in evaluating safety in very young patients and judging them in an uncontrolled trial. Thus, a clear picture of safety could not be derived from these data and a high degree of uncertainty remained. No fatal events related to therapy were observed; but this may also have been related to the small number of patients studied or to the highly experienced study centres administering the treatment.

Furthermore, the results of the GCP inspection raised concern as regards the completeness and reliability of the AE/SAE (serious adverse events) that were reported. It was therefore difficult to have a reasonably comprehensive estimate of the risks of unfavourable effects related to the treatment.

Benefit-risk balance

Importance of favourable and unfavourable effects

The most devastating consequence of UCD is cerebral damage caused by hyperammonaemia. The prevention of cerebral damage would be the most clinically relevant endpoint. Eventually these children die from complications of disease, thus mortality would also be a clinically relevant endpoint.

However, the prevention of the occurrence of hyperammonaemia or the decrease in the level of ammonia reached during hyperammonaemic events were considered relevant clinical outcome measures that were investigated in the study. Tight control of ammonemia level with decreased incidence of hyperammonaemic events are needed to improve the condition of patients with urea cycle disorders and to allow them to reach, without neurological damage, the point in time where OLT is feasible. Therefore, control of hyperammonaemic events would likely contribute significantly to the final outcome of these patients.

Data from the ^{13}C ureagenesis assay, which measures directly the activity of the enzyme in the liver has no direct clinical relevance but could in principle be an independent objective measure of drug activity. If changes of sufficient magnitude were observed this could be of importance to ascertain the benefit of the product.

Other changes that are observed and that could serve as indicators for efficacy e.g. glutamine elevation are of secondary importance and do not independently contribute to the benefit considerations.

The safety data, although only defined very coarsely with a high degree of uncertainty demonstrate that the major risks associated with the product are related to the procedure of administration and to a lesser degree to the immunosuppressive treatment.

Benefit-risk balance

In the opinion of the CAT no sufficient demonstration of favourable effects has been achieved. The trends observed in analyses of incidence of hyperammonaemia, height of hyperammonaemia, deaths, time to hyperammonaemic events of varying severity were in totality not sufficient to demonstrate a clinically relevant benefit.

The results from the 13C ureagenesis assay also lacked demonstration of clinical relevance, even if some changes were observed pre/post-treatment in individual patients and exploratory statistical analyses.

Unfavourable effects were observed and related to surgery, administration procedure and immunosuppression.

Therefore the benefit-risk balance is unfavourable.

Discussion on the benefit-risk balance

The uncertainties around the favourable effects observed, the lack of robust demonstration of their clinical relevance and the questionable effects size did not allow to conclude on a reasonably sufficient demonstration of benefit. The manifold analyses submitted were not able to alleviate these concerns.

Uncertainties around the pharmacodynamic endpoints in the form of the 13C ureagenesis were also not resolved.

Benefit/risk assessment of medicinal products is ideally based on randomised controlled studies which represent the best option to prevent bias and enable the evaluation of efficacy and overall risk of a product. When faced by rare diseases realisation of such RCT including a sufficient number of patients may not be possible. Assessment then relies on single arm trials with the inherent difficulties in analysis and interpretation. It is unlikely that marginal effects can be detected from small cohorts of patients based on these methodological constraints.

Further collection of efficacy and safety data within ongoing or future trials is required to broaden the experience and to gather reliable data that would be compelling enough to support a robust benefit/risk assessment.

In conclusion, the CAT considered that efficacy has not been demonstrated with reasonable certainty, even in the context of conditional MAA with limited data, and it is possible that observed trends as regards reduction of hyperammonaemic events are chance findings based on a comparison to historical control with too many biases; therefore the CAT considered the benefit-risk balance negative.

The CHMP endorsed the CAT conclusions on Benefit Risk balance of Heparesc based on the assessment described above.

4. Recommendations

Similarity with authorised orphan medicinal products

Not applicable

Outcome

Based on the CAT review of data on quality, safety and efficacy for Heparesc in the treatment of :

“paediatric patients from birth to less than 3 years of age suffering from severe urea cycle disorders (UCD) for whom orthotopic liver transplantation is considered a treatment option at a later timepoint.

Underlying enzyme defects may be carbamoylphosphate synthetase 1 deficiency, ornithine transcarbamylase deficiency, argininosuccinate synthetase deficiency (citrullinaemia type 1), argininosuccinate lyase deficiency (argininosuccinic aciduria), or arginase deficiency (hyperargininaemia)”,

the CHMP considers by consensus that the efficacy of the above mentioned medicinal product is not sufficiently demonstrated, and, therefore recommends the refusal of the granting of the conditional Marketing Authorisation for the above mentioned medicinal product. The CHMP considers that, whereas:

- Clinical relevance of the observed changes in 13C ureagenesis assay has not been demonstrated and there are still uncertainties regarding the variability of the data gathered from this assay.

- Efficacy has not been demonstrated with reasonable certainty (even in the context of conditional MAA with limited data), and it is possible that observed trends as regards reduction of hyperammonaemic events are chance findings based on a comparison to historical control with too many biases
- Overall there were many methodological issues in the studies conducted and a lack of consistency in the data presented. Data were analysed post-hoc, and many sources of bias were present. In view of the results presented a strong endpoint was missing, and correlation between the different endpoints used was not demonstrated

The CHMP is of the opinion that pursuant to Article 12 of Regulation (EC) No 726/2004, the efficacy of the above mentioned product is not properly or sufficiently demonstrated and the overall benefit/risk balance is unfavourable.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, pharmacovigilance system, risk management plan and follow-up measures to address other concerns as outlined in the list of outstanding issues cannot be agreed at this stage.

Furthermore, the CHMP, in light of the negative recommendation, is of the opinion that it is not appropriate to conclude on the new active substance status, similarity, market exclusivity and/or the significance of paediatric studies at this time.

5. Re-examination of the CHMP opinion of 25 June 2015

Following the CHMP conclusion that Heparesc was not approvable because its efficacy has not been demonstrated with reasonable certainty, the applicant submitted detailed grounds for the re-examination of the grounds for refusal.

Following a request from the applicant at the time of the re-examination, the CHMP convened an Ad Hoc expert Group inviting the experts to provide their views on the CHMP grounds for refusal, taking into account the applicant's response.

5.1. Detailed grounds for re-examination submitted by the applicant

The applicant presented in their submission the following grounds for re-examination:

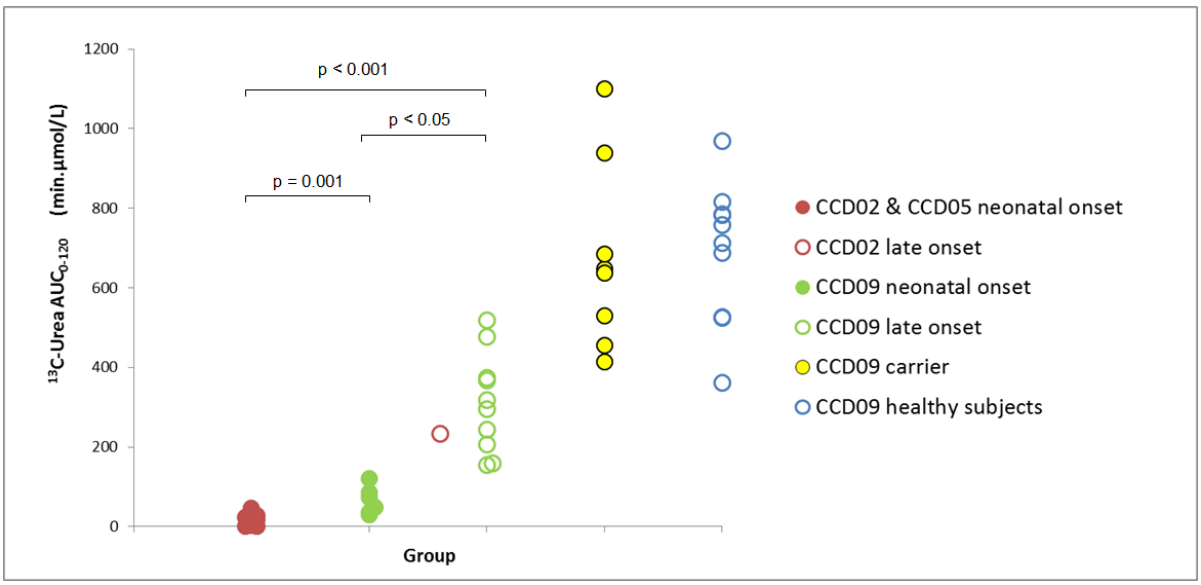
Ground #1

• Clinical relevance of the observed changes in 13C-ureagenesis assay has not been demonstrated and there are still uncertainties regarding the variability of the data gathered from this assay.

Summary of the Applicant`s position:

The Applicant claims that validation has been demonstrated in responses provided in the initial assessment phase. Regarding the clinical relevance the Applicant presented data showing that the 13C-assay distinguishes between populations with different severity grades of UCD (including healthy volunteers). The applicant claims that in the same way the assay shows a clear difference in the ureagenesis capacity in the treated patients when post-treatment values are compared to pre-treatment values, and that as these increases can only be explained by a contribution of the infused Heparesc cells this serves as a proof that these cells do engraft in the recipient liver and remain functional for a prolonged period of time.

Figure 4: Ureagenesis capacity in healthy volunteers and patients with UCD of different severity grades

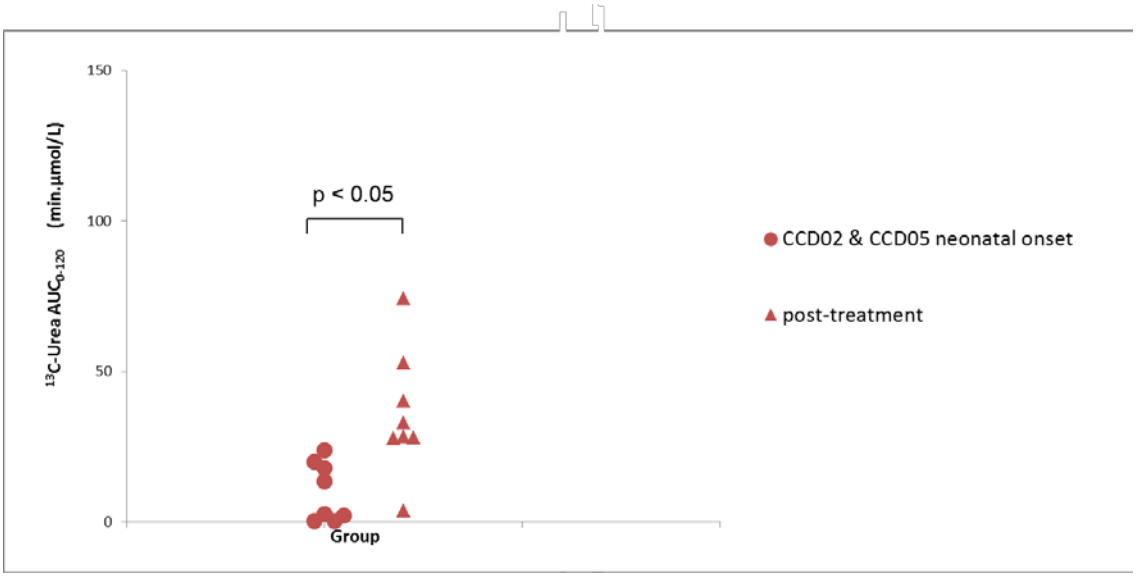


Using the AUC as primary measure the population of asymptomatic carriers of UCD mutations are clearly different from symptomatic patients (late onset and neonatal onset combined). The comparison of neonatal onset patients with the combined group of asymptomatic carriers and healthy volunteers shows a highly significant difference with $p<0.001$. Even the differences between the neonatal onset and late onset patients are statistically significant ($p<0.05$). So, although there appears not to be a clear-cut threshold value that determines clinical symptoms, the test provides reliable results for different patient populations. The plot shows that the neonatal onset patients are those with the lowest AUC-values and within this group the patients enrolled in studies CCD02/05 represent the most severely affected population.

In individual patients the test shows increases in the AUC values after treatment with Heparesc. However, in some patients there are fluctuations in the values when the test is done at several time points after treatment, which currently are not fully understood. Possible explanations include especially mechanisms of up- and down-regulation of the urea cycle. The Applicant therefore agrees that the test at this point in time should not be used as a method for day-to-day measurement of treatment effect; however, it is suitable to detect differences in populations and thus also differences when using the test for pre-/post-comparisons in the population of treated patients.

Figure 5 shows the max. AUC values measured after treatment compared to pre-treatment values for patients for whom pre- and post-treatment values are available.

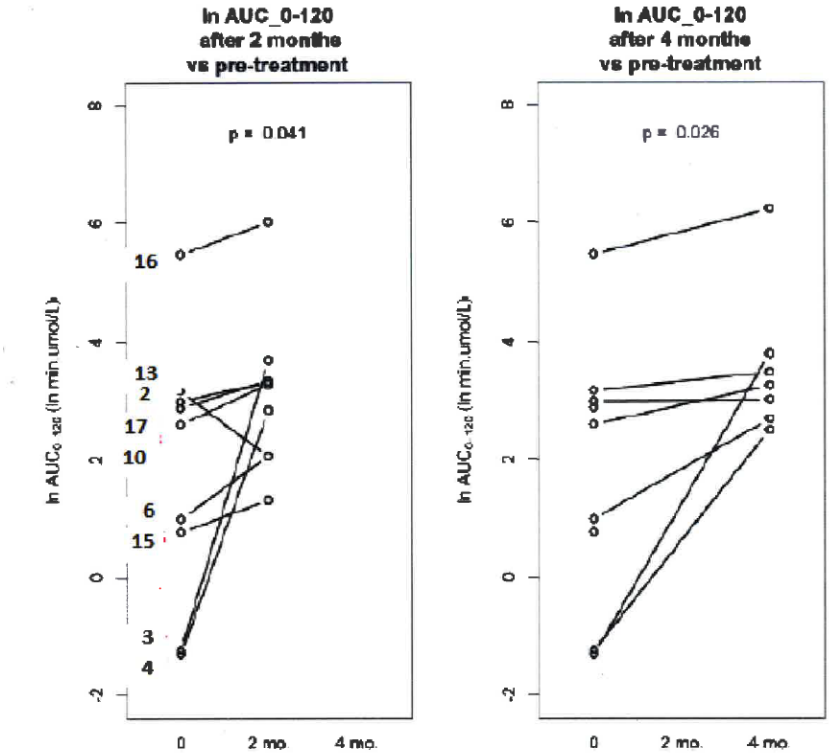
Figure 5: Max. ureagenesis capacity post-treatment compared to pre-treatment values for patients in whom both data are available



According to the graph there seems to be one clear treatment failure in whom AUC after treatment remains close to 0 (pat. 15), but in the overall population of treated patients max. AUC of 13C-urea as a measure of urea cycle capacity is markedly higher than before treatment. The difference is statistically significant ($p<0.05$). A direct relationship to clinical symptoms can not be expected here as there are no thresholds for clinical symptoms.

When looking at results at different time points (2 and 4 months after treatment) increases in ureagenesis capacity can be seen for most of the patients at both time points.

Figure 6: Ureagenesis capacity at 2 and 4 months after treatment in comparison to baseline



Ground # 2

• Efficacy has not been demonstrated with reasonable certainty (even in the context of conditional MAA with limited data), and it is possible that observed trends as regards reduction of hyperammonaemic events are chance findings based on a comparison to historical control with too many biases

Summary of the Applicant`s position:

In the Grounds for Re-examination the Applicant presented clinical efficacy data based on individual case descriptions, supported by statistical analysis. From the total of 20 patients enrolled in studies CCD02/CCD05, the applicant presented four patients in whom metabolic stabilisation for around 300 days was achieved; bridging to successful OLT was achieved in three of those four patients.

Further three patients who experienced the typical initial hyperammonaemic crisis shortly after birth but for whom no documentation of additional pre-treatment hyperammonaemic events was available, presented with a period of metabolic stability after liver cell infusion, with ammonia levels around the normal range. The periods of metabolic stability lasted between 162 and 532 days. All three patients could be successfully bridged to OLT.

The applicant claims that the very favourable clinical outcomes have to be regarded as a result of the clinically relevant substitution of the UCD gene defects through effectively engrafted allogenic liver cells. The remaining 8 patients presented with only short periods of metabolic stability after LCI or with no discernible treatment effect

Description of Treatment Effect in CCD02/CCD05 Patients

From the total of 20 patients enrolled in studies CCD02/DDC05, 17 received the full liver cell dosage of six daily treatments. In two of the remaining patients the LCI was terminated due to a catheter dislocation during or after the first infusion (18 and 19) and the last remaining patient did not receive any LCI due to elevated PVP combined with signs of portal vein abnormalities (20).

Of the 17 patients with full liver cell dosing, 15 had neonatal onset type OTCD, CPS1D or ASSD. These patients are summarized in Table 2. Another female patient had late onset type OTCD (16) and one patient 17 presented with the initial hyperammonaemic crisis after the first 7 days of life in combination with a peak ammonia level <500 µmol/l, thus not fulfilling the strict criteria for the integrated analysis.

Of the 15 neonatal onset patients with full dosing, 7 presented with a prolonged period of stabilized ammonia after Heparesc infusion. They had ammonia values in the normal or near normal range for about 300 days with tolerance of catabolic triggers and without development of hyperammonaemic events. According to the duration of the periods of stability, the availability of increased pre-treatment information and the frequency and severity of events, the following patient groups can be determined:

Group 1: Four patients with prolonged stability periods following LCI and documented pre-treatment instability (1, 2, 3, 4),

Group 2: Three patients with prolonged stability periods following LCI but insufficient pre-treatment instability documentation (5, 6, 7)),

Group 3: Eight patients with no evident benefit following LCI or short observation periods (8, 9, 10, 11, 12, 13, 14, 15), either terminated by early elective OLTs, severe treatment errors, or the inability to tolerate catabolic trigger factors.

For the four patients in Group 1, data on the metabolic situation before LCI were available. The course of the disease after liver cell infusion can thus be compared to the period prior to LCI.

Table 12: Individual patients' summary - patients with neonatal onset disease and full dose treatment

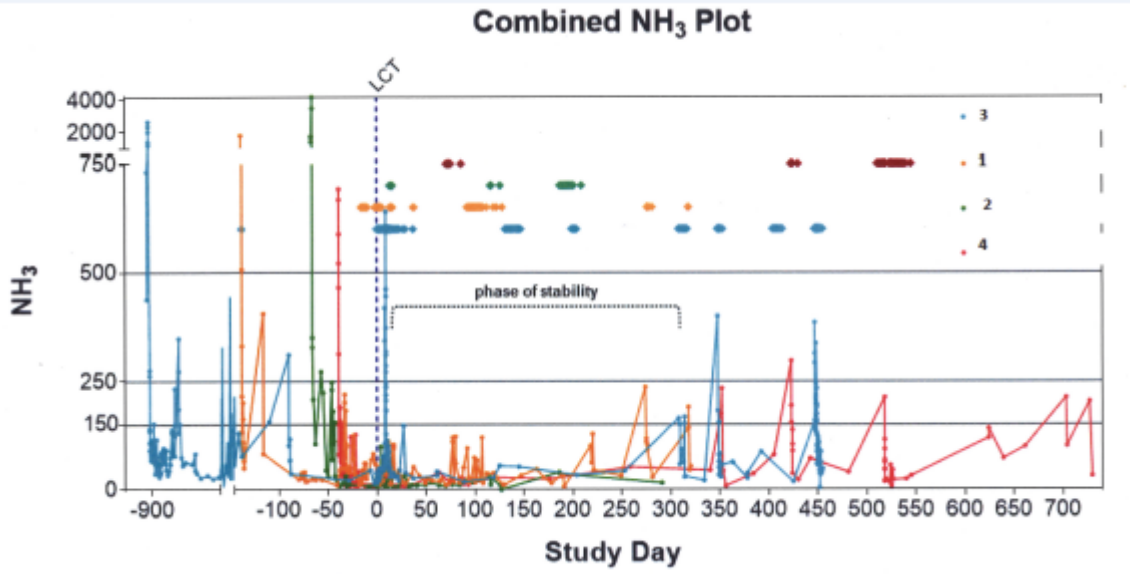
Patient nr / Disease	Peak ammonia level in Pre-LCI HE [after initial HE] (day of life)	Age (days) at first LCI (study day 1)	Stable after LCI until study day (ammonium peak value)	HEs after LCI, begin of HE at study day*			OLT at study day
				mild	moderate	severe	
Successful LCI (Group 1 and 2)							
1 OTCD	406 µmol/l (26), 220 µmol/l (109)	143	274 (237 µmol/l) (fever, in Dublin)		274		354
2 OTCD	272 µmol/l (14), 248 µmol/l (25), 155 µmol/l (28)	72	209 (OLT)				209
3 OTCD	Repeated HEs with increasing intensity during last year before LCI	907	8 (640 µmol/l) (adenovirus infection) Afterwards stable for 300 days	308	348 448	8	No OLT
4 ASSD	Fluctuation around 130 µmol/l	46	351		351 423 518 727		After study end at age of 34 months ('study day' 987)
5 OTCD	Not documented	37	162 (177 µmol/l) (vomiting)	162			176
6 ASSD	Not documented	33	211 (153 µmol/l)	211			253
7 ASSD	Not documented	89	532 (OLT)				532
Less favourable/no benefit LCI (Group 3)							
8 OTCD	Not documented	61	46 (259 µmol/l) (viral inf. in Montreal)		46 59 74		78 (large for size organ, death)
9 ASSD	Not documented	38	70 (213 µmol/l) (*Pneumocystis jirovechii*)		70		130 (drowned donor), re-OLT 136 (sepsis, death)
10 OTCD	313 µmol/l (23)	69	35 (>2000 µmol/l) (accidental high protein dose)	49	180	35 149	232
11 OTCD	None	1	4 (during LCI) (686 µmol/l), 11 (350		11	4 18	107

Patient nr / Disease	Peak ammonia level in Pre-LCI HE [after initial HE] (day of	Age (days) at first LCI	Stable after LCI until study day (ammonium peak	HEs after LCI, begin of HE at study day*			OLT at study day
			µmol/l), 18 (1190 µmol/l) (Staph. aureus infection)				
12 CPS1D	200 µmol/l (19)	29	15 (234 µmol/l)	15 36 48 51 63 86	 42 69		99
13 ASSD	Not documented	92	53 (180 µmol/l), 162 (294 µmol/l) Induced by norovirus	53	162 219		After study termination at an age of 696 days ('study day' 605)
14 OTCD	Not documented	84	24 (434 µmol/l)		25	69	No OLT, death at an age of 166 (`study day` 83).
15 OTCD	Not documented	101	14 (248 µmol/l)	7 96			105

Patients with evident treatment effect and available pre-treatment documentation of metabolic instability (1, 2, 3, 4)

Metabolic instability prior to LCI, manifested by a number of hyperammonaemic events likely based on intolerance of catabolic trigger factors, enabled a pre- to post-treatment comparison in patients 1, 2, 3 and 4. In comparison to the unstable period prior to LCI, all four patients presented with an effective metabolic stabilization after liver cell therapy that lasted about 300 days. In three of the four patients, this period was followed by a period of re-appearing instability, further corroborating the ability of the donor cells to at least partially restore liver function. During the period of stability, the patients' ammonia levels generally remained constantly within the normal range, even in the presence of typical catabolic triggers (Figure 7)

Figure 7: Patients with evident treatment effect - combined ammonia plot



Coloured dots: Adverse events/potential catabolic triggers.

All four patients showed an adequate protein intake resulting in normal growth and development (apart from any damage inflicted by the pre-treatment crises).

Three of the four patients could be successfully bridged to OLT. The gained time window with metabolic stability and avoidance of brain damage could enable further growth of the patient and thus increase the chance for a successful OLT. In addition, the stable period could be used to identify a donor organ with best medical fit. At the time of OLT, all children were in overall good clinical condition.

In summary, metabolic stabilisation for approximately 300 days was achieved in all four patients; bridging to successful OLT was achieved in three of the four patients. The applicant claims that the very favourable clinical outcomes have to be regarded as a result of the clinically relevant substitution of the UCD gene defects through effectively engrafted allogenic liver cells.

Patients with evident treatment effect but no available pre-treatment documentation of metabolic instability (5, 6 and7)

The three patients who experienced the typical initial hyperammonaemic crisis shortly after birth but for whom no documentation of additional pre-treatment hyperammonaemic events was available, presented with a period of metabolic stability after liver cell infusion, with ammonia levels in the normal or near normal range. The periods of metabolic stability lasted between 162 and 532 days.

All three patients could be bridged to OLT, with the time period to OLT sometimes depending on factors unrelated to the metabolic status of the patient. For example, the first patient being treated in CCD02 study (5) was brought to OLT rather early after liver cell infusion (on study day 176). For none of these patients an early OLT due to deteriorating ammonia levels was urgently indicated.

Patients with no evident benefit from LCI (8, 9, 10, 11, 12, 13, 14, 15)
Two patients (8 and 9) showed a single event of mild or moderate hyperammonaemia after at least 35 days of metabolic stability following LCI. Both patients received an early OLT afterwards and died of complications associated with the OLT. One patient (10) received an accidental bolus of a potentially lethal protein intake that caused significant neuronal damage and led to recurrent episodes of epilepsy and associated hyperammonaemic events. In general, these three patients showed at least a brief period of metabolic stability after LCI, however, the longer-term treatment effects could not be determined because the periods after LCI were either terminated by elective OLT or interrupted by severe poisoning.

The five remaining patients showed no apparent benefit from Heparesc treatment. In case of patient 11, the observed metabolic instability could be explained by the interference of the cell infusion with the initial decompensation. In addition, this patient has received all six cell infusions into the left liver lobe only. Patient 14 was post-LCI diagnosed to have structural liver disease, a condition highly likely to preclude donor cell engraftment.

Besides patients 8 and 9, who died after OLT, four of the remaining six patients underwent successful OLT.

Ground # 3

- Overall there were many methodological issues in the studies conducted and a lack of consistency in the data presented. Data were analysed post-hoc, and many sources of bias were present. In view of the results presented a strong endpoint was missing, and correlation between the different endpoints used was not demonstrated.

Summary of the Applicant`s position:

Given the very small patient number, the Applicant presented, as supportive evidence statistical evaluation of the relevant clinical endpoint 'time to hyperammonaemic event' in the clinical studies CCD02/CCD05 in comparison to a selected historic control group (CCD10). Although it is acknowledged that this approach has certain limitations, the applicant arguments that this approach is conservative in several ways (CCD10 population not particularly severe, enrolment bias to the disadvantage of the treatment group, elimination of all deaths and early events in the control group due to the methodology used) and that the selection of the relevant control group without the mild cases is well justified and confirmed by the 13C data. The applicant claims there is a clear difference in the time to event analysis for both, the moderate as well as the severe hyperammonaemic events, meaning that treated patients have a clearly higher chance of receiving an OLT without experiencing further such hyperammonaemic events as compared to control. The applicant is of the opinion that the extent of the difference is clinically relevant, and that given the orphan indication, the individual clinical data and the statistical analysis are consistently showing efficacy.

Efficacy information derived from detailed analysis of individual cases is supported by a comparison of patients treated with Heparesc to a historic control group. A controlled clinical trial, even more a blinded randomized parallel group study, is not feasible for various reasons, in particular the low incidence of the disease. In the Applicant’s view the same holds true for a cross-over design as has been used with scavenger drugs in UCD.

Pre-post comparisons are important and have been done for the individual cases as much as possible and shows a stabilisation of ammonia values in a number of patients that had been unstable, i.e. showing repeated ammonia peaks before study treatment. However, as many patients were referred to the treatment centres from other hospitals, data on the period before study enrolment are limited and also not standardised in terms of treatment and data capture. Also, the majority of patients have been treated

very early (almost 75% at ≤ 3months of life, 40% at ≤ 2 months of life). Therefore a systematic statistical pre-post analysis is not possible.

A historical control group therefore is seen as the only possibility to statistically compare the clinical course of patients treated with Heparesc to patients treated according to state of the art current treatment. Therefore a separate study (CCD10) has been set up to collect data on neonatal onset UCD patients from major European treatment centres (N=63).

In order to provide the best possible comparison, data from the studies CCD02 and CCD05 (treatment) as well as CCD10 (control) have been evaluated in an integrated analysis for which a statistical analysis plan (SAP) (Version 2.0) had been prospectively established.

Table 13: Data sets analysed

Patients	HHLivC	Control
Safety Set	**20**	**63**
Exclusion from the FAS		
▪ No initial ammonia value >500 µmol/l **and** no evidence of prenatal diagnosis or documented family history and immediate treatment	2 (10.0%)	16 (25.4%)
▪ Diagnosis of UCD not provided	–	1 (1.6%)
▪ LCT in Control	n.a.	1 (1.6%)
▪ Never received any LCT in HHLivC	1 (5.0%)	n.a.
Full Analysis Set (FAS)	**17 (85.0%)**	**45 (71.4%)**
Exclusion from the PP / RC		
▪ Death in first hyperammonemic event	–	12 (19.0%)
▪ Patients with mild disease	–	14 (22.2%)
▪ Start of LCT without hyperammonemic event	1 (5.0%)	n.a.
▪ Pre-existing structural liver disease (liver cirrhosis, portal hypertension)	1 (5.0%)	–
▪ LCT not completed	2 (10.0%)	n.a.
▪ Clinically significant treatment errors occurred	1 (5.0%)	–
Per Protocol (PP) / Relevant Control (RC) Set	**12 (60.0%)**	**19 (30.2%)**

Source: Table 9.1-1 in the integrated analysis report, Version 2.0

Nevertheless, for the comparison of patients in the treatment studies CCD02/05 with a matching control group from CCD10 a number of factors influencing the results have been major issues in the review of the Heparesc application, most importantly

- the potential of an enrolment bias in studies CCD02/05 with the question whether a population of less severely affected than patients in CCD10 was more likely to be enrolled in the treatment studies,

- the selection of the relevant control set from the CCD10 database,

- the effect of event definitions (start and end of a hyperammonaemic event) in the statistical analysis plan, especially the so called 'early events' .

The Applicant is of the opinion that these concerns have been adequately addressed and that the primary analysis as described below represents a conservative approach regarding these issues for the following reasons:

Control Population (CCD10) and Matching (Bootstrap Methodology)

Overall patients in the CCD10 control study seem to represent a patient population less severely affected than average neonatal onset UCD patients at least in terms of mortality:

In CCD10, 19 of 63 patients enrolled died, 16 of them in the neonatal period, thereof 12 during the initial event. (Three further patients died after more than 1 year). This represents a neonatal as well as 1 year mortality of 25%, which is within the spectrum of mortality rates from the studies found in literature; however it certainly is at the lower end of observed mortality rates In addition two further measures in the statistical evaluation constitute conservative approaches with regard to the patient selection for the analysis:

1. All patients from CCD10 (control) who died during the initial crisis have been excluded from the relevant control set ('early deaths', N=12) as these patients probably would also not have been enrolled in the treatment study because they died too early after birth.

2. As a direct matched pair analysis proved to be not feasible due to the small sample size, a bootstrap approach has been used in order to provide best possible matching of treated and control patients on an individual case level. Two co-variables have been used for matching, i.e. UCD subtype and patient age at start of treatment. This means that for every individual pair comparison data of the control patient have only been used from the day that matched the age of the treated patient at day of treatment. As the youngest age at treatment start in the PP group was 28 days this adjustment results in the omission of all events before day 28 in the control group and thus all the early events (including deaths) that have been challenged during the review process are excluded from the analysis.

With regard to mortality this analysis therefore represents the most conservative approach possible. Of the remaining patients most have been treated conservatively and only 7 patients from the RC set (37%) and 11 patients overall (17%) received a liver transplant during the observation period in CCD10.

Treated population CCD02 and CCD05

In contrast, in the treatment studies CCD02/05 15 patients (75%) received a liver transplant within the 24 months study period. This shows again the severity of these cases as they have obviously not only been 'considered for OLT' but indeed did receive a liver transplant. In addition all patients had a genetic mutation categorized as severe or likely severe. The pre-screening log also lists a number of cases where the reason for not enrolling the patient was documented as 'too stable to be enrolled', showing that treating physicians did not refer patients for study enrolment if they expected them to be manageable with conservative treatment. For all these reasons it is highly unlikely that there was an enrolment bias in favour of the treatment group, on the contrary the data above clearly point into the direction that more severely ill patients have been enrolled in CCD02/05 as compared to the mix of patients in CCD10.

In addition data from a functional in vivo test of the ureagenesis capacity ('13C-assay') in individual patients as well as healthy volunteers clearly show that patients treated in CCD02/05 had the lowest ureagenesis capacity of all groups investigated. Looking at the overview data in Figure 4 it is clear that in terms of ureagenesis capacity (pre-treatment values) patients in CCD02/05 represent the most severely affected patient population and already the comparison with the neonatal onset patients in study CCD09 shows a statistically highly significant difference (p=0.001) despite the small patient number Four of the patients in CCD02/05 even had pre-treatment values close to zero. No values in this range have been observed in CCD09 and it is therefore to be considered unlikely that patients with this severity of disease could have survived in a relatively stable clinical state without liver cell treatment and/or liver transplant as did the patients in CCD09.

Exclusion of 'Mild Cases' in CCD10

For patients that were considered less severely affected some treating physicians took a 'wait and see' approach and did not refer the patients directly to the treatment centres or enrol the patients into the

study, as also documented in the pre-screening log ('too stable to be enrolled'). There is even one patient (17) who after the initial crisis was considered to be too stable to participate in the study but at an age of about 2,5 years, when suffering from new hyperammonaemic events (thus becoming 'unstable'), was then enrolled.

For these reasons, when defining the per protocol control group ('relevant control'), cases were excluded who were completely stable without any hyperammonaemic event for at least 6 months after the initial crisis, as there is a high likelihood that in general such cases would not have been referred to the treatment centres.

'Early Events' in the Control Group

Another point that had been challenged during the review process were the early events observed in the control set and indeed for several of these events it can be considered probable that these still should be regarded as part of the initial crisis. In addition, an approach matching treated and control patients on an individual level according to age and UCD subtype had been recommended. As described above the statistical methodology of the bootstrap analysis using age and subtype as cofactors for matching takes care of both of these issues as it matches individual patients according to their UCD subtype as well as age at treatment. This means that for every treated patient the data for the matching control are only considered from the age that aligns to the treated patient. As the youngest age at treatment in the per protocol group was 28 days all events in the control group that happened before day 28 were omitted from this analysis, thus eliminating all deaths and all early hyper-ammonaemic events. Again this represents a conservative approach as it eliminates these early events only in the control group.

Efficacy Results

Avoidance of hyperammonaemic events, especially moderate and severe hyperammonaemic events is a primary objective in the treatment of UCD patients; time to event analysis is an appropriate analysis to investigate the effect of Heparesc. The applicant presented the results of the bootstrap analysis for the severe and moderate hyperammonaemic events. In addition to the median result (Q2) for the control group, the 25% quartile (Q1) and 75% quartile (Q3) for the probability of the event at each time point were plotted ; the applicant claims that these show the robustness of the analysis as even the 25% quartile is clearly different from the curve for the treatment group (HHLivC).

The Applicant pointed out that in this analysis there is one effect that goes to the advantage of the treatment group. The only patient who suffered a severe hyperammonaemic event in the treatment group during the study period (640 µmol/l at study day 8, two days after completion of liver cell infusion) was patient 3. The hyperammonaemic event was most probably due to an adenovirus infection with pneumonia and viral sepsis. The hyperammonaemic event was quickly controlled and thereafter the patient was very stable for a period of about 300 days. It has been questioned by the rapporteurs whether the cells would already be fully functional before 2 weeks and therefore the relevance of this event may be questioned, however in general it should of course be counted in the Kaplan-Meier analysis. The reason that this patient was not included in the bootstrap analysis was that the patient was already 30 months (906 days) old at enrolment and start of treatment and that there was no matching control patient in CCD10 which is why the bootstrap analysis omits the data for this patient. If this event were included, the overall difference between treatment and control is still clearly maintained.

In the individual case reports for a number of patients a phase of stability without any hyperammonaemic episode after treatment has been described that lasted for about 300 days to one year. This can be regarded as a relevant period of time that allows the children to grow to a size and weight that allows for transplantation with a higher probability of success. It also allows for a period of waiting for a suitable organ. When looking at the bootstrap data and comparing the treated population with the median curve for control this would mean that the probability of experiencing at least one severe hyperammonaemic

event while waiting for an OLT is 0% in treated patients (8% including patient 3) versus 33% (for 300 days) or 39% (for 1 year) in the control group. The probability of experiencing at least one moderate event is 30% (36% including patient 3) for treated patients, for both 300 days and one year while for the control patients it is 80% (within 300 days) or 87% (within 1 year).

The Applicant is of the opinion that the results of these statistical analyses clearly support the conclusions from the individual cases reported and that, given the long term consequences (irreversible damage) of severe hyperammonaemic events to the patients, these differences should be regarded as clinically relevant.

In the view of the Applicant and given the orphan indication the individual clinical data and the statistical analysis are consistently showing efficacy.

In addition to the grounds for re-examination the applicant presented at an oral explanation on 6 October and 15 October 2015.

5.2. Report from the Ad Hoc expert Group:

Following the receipt of the detailed grounds for the re-examination, the CAT/CHMP convened an Ad Hoc expert Group on Heparesc inviting the experts to provide Responses to the CAT List of Questions, and in addition their comments on the Grounds for negative opinion, taking into account the grounds for re-examination submitted.

1. **The expert group should comment on how comparable the Heparesc treated patient population (N=20) and the historical control group (n=63) are with regard to disease severity and mortality. Please also consider the chosen exclusion criteria which led to the definition of the patient sets for the integrated analysis (per protocol (PP)/relevant control (RC) set).**

The experts were of the opinion that it is very difficult to find a good historical control. Over time, standard of care has improved and more treatment options became available. Treatment change over time is important in matching evidence; this cannot be taken sufficiently into account for the analysis of the Heparesc data. The Heparesc treated group seems to have more severely affected patients than the historical control group, and historical controls were older than the Heparesc treated group. Furthermore, standard of care (SOC) provided currently is better than SOC received by the control group.

The main challenge is how to characterise mild patients (and how they were taken out of the control group). If a patient`s ammonia levels are well under control, this does not necessarily equal mild disease, but may be due to current treatment. Also, some patients have liver disorder which does not recover, regardless of recovery of ammonia levels. The experts questioned whether this was considered. Growth state, protein tolerance and overall metabolic stability are important aspects to look more realistically into severity of UCD.

The key issue with patient selection is that patients were treated very early on, so it was not yet known at that time whether these patients would have spontaneously stabilised without liver cell infusion. The honeymoon period after initial hyperammonaemia crisis is well known (sometimes very long, up to 6 months or a year). A trigger may be found (too high protein intake, infection,…), but not always is a trigger found. The patient group possibly had a more severe presentation than the controls (based on ureagenesis assay results).

It was also mentioned that the E-IMD registry is being established for collecting information on patients with intoxication type rare diseases such as UCD, and might help address the problem of control groups.

2. **What do the experts consider to be the most relevant endpoint, and the most important evidence of efficacy demonstrated for Heparesc? Please also comment on the evidence**

observed for engraftment and allogeneic cell activity of Heparesc.

There were some mixed views on this point. One expert said that he believes there is no clear correlation between the degree of residual ureagenesis and the clinical condition, despite the fact that there is a reasonably good overall correlation between **ureagenesis** assay and urea cycle capacity. However, the reliability of the assay for low degrees of residual ureagenesis (test is not fully validated) and of the results collected in the studies presented have not been sufficiently shown. The experts doubt from the data presented whether ureagenesis can be used as a biomarker for follow up of patients with very low ureagenic capacity, as is the case for severe UCD patients. In principle, ureagenesis would be of particular interest in patients with very low levels of ureagenesis (e.g. to measure an increase from 0% to 2 % of normal levels), but the experts are not confident that the test can measure these very low levels very precisely. The relevance of small changes in ureagenesis assay results for patients with a baseline level of 5% is therefore doubted. The evidence provided demonstrated poor correlation between changes in ureagenesis and clinical status. It was raised that OTC patient 3's "stable period" coincided with low ureagenesis activity and then hyperammonaemic episodes recurring when ureagenesis improved. The ureagenesis results were therefore not a convincing indicator for clinical effect of infused cells.

Biological variability also makes it difficult to evaluate **clinical endpoints**. One of the objectives of treating UCD patients is reaching good cognitive development (for which (hyperammonemia)/hyperglutaminaemia would be a parameter), as well as physical development (which could be measured by protein tolerance, and clinical presentation of one of the parameters, growth). The importance of clinically relevant data on cognitive development was stressed from the perspective of the patient representative.

The experts mentioned that cognitive assessments in young infants are difficult (there is no validated scale) and it is not possible to measure cognitive development in neonates, but developmental data can be collected to demonstrate whether neurological outcomes are likely to be better with the intervention. The briefing document from the company making the case for medical need for improved therapies included natural history outcome data showing only 13% with normal development at 1 year. This demonstrates that it is possible to collect some early indication of effect.

Overall, it would have been necessary to substantiate these cases with better evidence, not only post treatment but also pre-treatment. It was questioned by the experts, whether the cell infusion can have real impact on cognitive outcome. There was also a comment that it would be useful to know the dosages used of the scavenger drugs (as this might vary across Member States or centres despite clinical guidelines).

The key gap in the data presented is that available information is scattered, there are important intra-individual variations and for many patients pre-treatment data are lacking.

The experts find it difficult to attribute effects observed to the SOC (where often a similar pattern is observed), or to the administration of liver cells. **Engraftment** was not well documented. Investigation of all explanted livers (after OLT) would be a requirement for any patient receiving liver cell treatment, if going through further OLT. With some non-sense mutations or deletions, patients have no ureagenesis activity at all; for one such patient presented (patient3) the ureagenesis pattern is suggestive for some activity of infused liver cells (This type of mutation may have to be added to the inclusion criteria). However, long stable periods without the cell infusion makes it difficult to judge whether the 300 day stable period after LCI (liver cell infusion) is due to SOC (goes on throughout the procedure) or due to the cells. The evidence in support of allogeneic cell activity is considered very questionable.

3. **Taking into account the extreme rarity of UCD, are the experts of the opinion that the presented individual case reports convincingly support the efficacy of Heparesc in the proposed indication, or in a subpopulation thereof?**

For one single patient (3; null mutation) treatment with Heparesc showed a stable period after the LCI. However, a similar long stable period was seen prior to inclusion in the study. The assay indicated improvement in ureagenesis (0% D0 → 5.8% D50) in this patient who would be expected to have no activity in the absence of infused cells, suggesting a change related to the therapy. However, the ureagenesis activity then fell back to 0% by approx D200 before rising again to 3.1% by approx D425 when metabolic control was deteriorating; the opposite of what one would predict clinically. It would be desirable to identify this subpopulation of patients with null mutations and obligatory zero level of ureagenesis to assess the effects of treatment with liver cells. There is no clear correlation between ureagenesis measurements and HE in the other patients, and long stable periods were observed pre-treatment in several patients. Overall there is some indication of an effect, but whether it is due to the LCI the experts are not convinced based on the data shown to date.

The proposed indication of very early treatment of most severe patients is questioned; see discussion on honeymoon period after initial hyperammonemic crisis.

4. **What is the potential impact the Heparesc treatment may have on potential success or failure of a later OLT (orthotopic liver transplantation) in the Heparesc treated population?**

There is no data that would be cause of concern for a negative impact on outcome of later OLT. The factors regarding immunosupressants (IS) are acceptable as IS will also be needed when liver transplantation takes place. The main difficulties could arise from damage to the portal vein, although in the clinical trial no such problems were reported. One expert asked, whether there has been signs of ascites in the treated patients (suggesting too high portal vein pressure) in line with the ascites observed in the studies in rabbits.

On the other hand, nutritional state of the patient at time of OLT influences the success of transplant. Liver cell treatment could offer the possibility to buy some time before transplantation, by means of improving protein intake and, therefore, enhancing lean body mass.

5. **What is the experts´ view on the safety of the Heparesc administration including immunosuppression, and specifically, the risk of adverse effects related to the use of catheters in the portal venous system in small infants (related to catheter placement, cell infusion and continued presence over six days of treatment)?**

From the safety point of view the experts mentioned that issues with the portal vein are always a possibility after vena porta catheterisation, which may lead to the need for immediate transplantation. It will be very important that administration occurs only in specialised centres, with high level of expertise in the administration technique. A hepatic transplant unit and metabolic team should be readily available.

With regard to risk of embolism, the surgical technique for administration is understood to include a procedure to prevent the injected cells from being shunted to the lungs.

6. **a. What is the extent of medical need beyond present standard of care in neonatal onset UCD patients taking into account different levels of severity? What is the current situation in Europe regarding OLT for children with metabolic disease such as UCD in different paediatric age sets (donor organ availability, time on waiting list, specialised transplant centres, expected outcomes, etc.)?**

   b. Based on the currently available data, could the experts see the role of Heparesc in current treatment approach of UCD, particularly would Heparesc fulfil an unmet medical need (and to what extent)?

6a. The experts confirmed that UCD is an area with unmet medical need. Differences in Europe were highlighted with regard to transplant waiting lists. For patients with rare diseases election for a transplant is very personalised; there are also regional differences in tendency to transplant. The patient representative sees a medical need, in that a small improvement in neurological outcome is very relevant;

however, the experts clarified that patients with neurological damage are often considered not suitable patients for transplantation. Some urea cycle enzymes are expressed in the brain, and therefore it is conceivable that their enzyme defects could have direct effects on the brain that might be unrelated to hyperammonaemia, although this is not actually known. The experts agree though that there is some degree of medical need beyond the present SOC for the patients with very severe neonatal onset CPS, OTC deficiency (boys) and citrullinemia (ASSD) to bridge patients to a later point in time, where liver transplantation can be performed. However this cannot be shown from the data presented. It was also concluded that this treatment, by itself, is not able to prevent neurological damage, nor is the first crisis preventable by this treatment. One expert mentioned that overall, outcome of liver transplantation is better for metabolic disease than for non-metabolic disease transplant indications.

6b.Patients with no enzyme activity at all, i.e. the most severe group of young UCD patients who are also entitled to transplantation, might be considered for the **principle** of this type of treatment as add-on to current SOC. The strength of evidence available for Heparesc so far is considered rather weak, and the experts would welcome further data collection to further investigate the principle.

The experts explained also that in many cases severe hyperammonaemia can be treated with currently available interventions. Aggressive haemodialysis in addition to conventional therapy remains the first treatment in case of such a crisis to remove high ammonia levels. Conventional therapy includes the use of ammonia scavengers supplementation and protein restriction. After hyperammonaemia crisis intervention, complete recovery and a long stable period under conventional SOC is often seen (honeymoon effects, discussed above). Only for some patients who have less good recovery (rising glutamine levels, instable metabolic state etc) and who would be in urgent need of OLT could liver cell infusion be considered as add-on to SOC, while awaiting appropriate patient weight (10kg) and while waiting for a liver transplant becoming available. Heparesc immediate liver infusion after an initial hyperammonaemia as presented in this application is a different concept from the current stream of thought.

Perhaps early hepatocyte infusion could focus on prospectively diagnosed male OTC patients with neonatal deaths in the family history with the aim of avoiding hyperammonaemic decompensation prior to cell infusion and the cells bridging to transplant within the first year.

In addition, the expert group commented on the grounds for re-examination submitted:

It was difficult to conclude from the data presented that there is sufficiently conclusive evidence of efficacy, or what is the most suitable moment for LCI, taking into account both clinical stability and availability for further OLT. The **concept** is very interesting and promising, but the experts are not convinced of the evidence seen for the current application.

The CAT and the CHMP have considered the outcome of the advice provided by the Ad Hoc expert Group.

5.3. Overall conclusion on grounds for re-examination

The CAT and the CHMP assessed all the detailed grounds for re-examination and argumentations presented in writing and in an oral explanation by the applicant and considered the views of the Ad Hoc expert Group.

Ground#1. Clinical relevance of observed changes in 13C-ureagenesis; variability of data gathered

The ureagenesis assay can be seen as a useful biomarker to indicate a functional improvement by measuring turnover of intraportal ^{13}C-actetate/bicarbonate administration and ^{13}C incorporation into subsequently synthesized urea. However, the reliability of the assay for low degrees of residual ureagenesis and of the results collected in the studies presented have not been sufficiently shown.

The data from both investigational sites were problematic. No quality assurance system was in place and only partial GLP requirements were fulfilled at the Children`s Hospital of Philadelphia (CHOP) laboratory; the method (absolute 13C bicarbonate determinations in plasma) at the second centre (INFAI laboratory) was not fully validated according to the recommendations set by the EMA guideline on bioanalytical method validation for quantitative concentration determinations in study samples from human studies (2011) and the observed average accuracy of 75.9% was too low to meet the acceptance criteria of 85 to 115% accuracy set by the guideline.

When comparing ureagenesis data generated in different patient populations with distinguishable severity of clinical disease to the changes observed in the treated patients these changes are only marginal and clinical relevance can hardly be attributed.

The link between ^{13}C uptake, incorporation into the urea cycle and subsequent ^{13}C-labeled urea formation is not straightforward. Other pathways feeding into the urea cycle could redistribute the labelled ^{13}C to other metabolites. Therefore, while the principal mechanism of action - that urea formation is dependent on functional liver cells - is agreed, quantifying the functional improvement would require a more comprehensive measurement of other possible metabolites as well.

The data presented show a clear correlation of urea synthesis (expressed as the ^{13}C urea $AUC_{0\text{-}120min}$) with different severity grades of UCD, with late onset patients having higher levels than neonatal onset patients. In the few patients where both pre- and post-treatment levels of ^{13}C urea $AUC_{0\text{-}120min}$ are available, an improvement of this biomarker was noted for most subjects both after 2 and 4 month, compared to baseline. However, such improvements, while measureable, seem marginal in several cases, with a pronounced effect only in patients with very low initial value, i.e. cases with neonatal onset. This would imply that a treatment in a severe subset might be of value to further investigate, but for other cases (and the exclusion criteria are currently somewhat elusive), the biomarker does not indicate a clear improvement.

There are other difficulties with interpretation of these data. One is the high fluctuation of the post-treatment values. This is not surprising, as many parameters like metabolic state, nutritional intake or physical activity could have a confounding influence. It is also quite likely that such variability could depend on the deficient enzyme involved, as e.g. OTCD and ASSD would result in accumulation of different metabolites. This makes it difficult to measure a day-to-day treatment effect, but would still make it suitable to detect differences between populations. Facing the high inter-individual differences also within different severity groups this would not even allow conclusive assumptions on treatment effects in such severity groups. The major uncertainty arises from the amount of improvement of the ^{13}C urea $AUC_{0\text{-}120min}$ levels. With ^{13}C urea $AUC_{0\text{-}120min}$ values in healthy subjects in a range > 600 min x µmol/L even the most severe patients with baseline values < 25 min x µmol/L (3-5% of normal) show as treatment effects not more than an additional 5 – 6%, this being still about 90% below normal values. It is agreed that clinically relevant improvements might well be far beyond this level, and the Applicant referred to publications, that show near normal growth and development with 10-15% of normal levels (Yudkoff et al 1980, Yudkoff et al 1996, Matsuda et al 1991, Ban et al 2001). Hence, in this setting of patients with severe UCD where the relevance criterion would have to consider any improvement of a fatally low level, such seemingly low AUC improvements might well be clinically relevant. And with patients starting at different baseline levels neither a threshold for a minimal percentage or absolute value of improvement can easily be defined. But definition of a clinically relevant improvement currently is not sufficiently established and would require confirmation by directly correlating data with clinical symptoms.

The Ad Hoc Expert group doubted from the data presented whether ureagenesis can be used as a biomarker for follow up of patients with very low ureagenic capacity (severe UCD). In principle, ureagenesis would be of particular interest in patients with very low levels of ureagenesis (e.g. to

measure an increase from 0% to 2 % of normal levels), but the experts are not confident that the test can measure these very low levels very precisely. The relevance of small changes in ureagenesis assay results for patients with a baseline level of 5% is therefore doubted. The evidence provided demonstrated poor correlation between changes in ureagenesis and clinical status.

Therefore, while agreeing that a correlation between urea synthesis and liver function is both likely and relevant information, data currently do not allow concluding from ureagenesis improvement (and lack of improvement) directly on clinical relevance.

Ground#2. Efficacy not demonstrated with reasonable certainty; reduction of hyperammonaemic events could be chance findings

As the Applicant´s responses were not organised in a way which addresses each of the three grounds for refusal/re-examination extensively and separately, a substantial part of the concerns expressed in Ground #2 (issues relating to the definition of the historical control group and the "integrated data analysis"), is therefore summarised and discussed under "Methodological issues" ground 3.

The Applicant stressed the importance of considering the individual patients´ reports - in view of the small number of patients and variable course of metabolic disease such as UCD - to assess treatment benefit; efficacy of Heparesc in UCD patients should be judged by achievement of a period of metabolic stability after LCI, characterised by the absence of significant (i.e. moderate and severe) hyperammonaemic events. This would allow for a normal development of the infants and delaying of OLT to a later age, when less procedural complications and better outcomes are expected.

The Applicant briefly summarised the individual case reports of patients with neonatal onset of UCD symptoms who received the full liver cell dosage on six consecutive days, which applies to 15 of the 20 patients included in the pivotal studies CCD02 and CCD05.

7 of the 15 patients had a favourable clinical course after LCI. Documentation was sufficient for a meaningful comparison of the clinical course pre- and post-LCI in 4 of the 7 subjects; they experienced a period of metabolic stability after LCI of around 300 days and 3 of the 4 received an OLT later on. Evidence of donor cell engraftment in the explanted liver documenting the success of LCI was obtained in 4 of the 7 patients (for 3 patients there is either no available information or they did not receive OLT until data cut-off date/ end of study).

In 8 of the 15 patients the benefit of LCI could either not be ascertained due to a short observation period after LCI (n=3) or no benefit was evident (n=5). In some of these patients the timing of LCI (on day 1 of life), adverse conditions for LCI such as presence of liver fibrosis and a major mistake in the diet could explain the failure to demonstrate benefit of LCI. In others frequent infections (probably due to immunosuppression following LCI) led to repeated bouts of hyperammonaemia and metabolic stability was not achieved.

Even if one were to accept these case reports as being appropriate and sufficiently informative for evaluating treatment effect in a very small patient population, the descriptive evaluation of the results shows a mixed picture. In more than half of the patients of this "Per Protocol" population a benefit of LCI could either not be ascertained or was absent. In 4 of 15 patients the data can be interpreted as supportive of a treatment effect of LCI leading to a period of reduced hyperammonaemic events and metabolic stability. No factors predicting a favourable/unfavourable outcome have been identified and given the small sample and the variable clinical course of UCD this is not unexpected.

A possible explanation for the metabolic stabilisation observed in the 4 patients after LCI (as opposed to their instable pre-treatment course) could be by an increase in enzymatic activity of the transplanted heterologous liver cells (and duration of the metabolic stabilisation of 300 days is in line with what is reported in the literature for liver cell transplants). Unfortunately the chain of evidence of the efficacy of Heparesc is incomplete; a well-documented increase in ureagenesis capacity pre- and post-treatment in

these patients (and ideally also evidence of enzyme activity in the explanted liver after OLT) is partly missing. Though the technical challenges of these investigations are acknowledged, this might have largely ruled out the possibility that the reduction of hyperammonaemic episodes in the 4 patients post LCI is a chance finding due to variability in the clinical course of the disease.

Overall, it would have been necessary to substantiate these cases with better evidence, not only post treatment but also pre-treatment. The experts find it difficult to attribute effects observed to the SOC (where often a similar pattern is observed), or to the administration of liver cells. Engraftment was not well documented.

In summary, even if one considers the rarity and severity of the disease, the evidence from these case reports is not considered sufficient for concluding on efficacy of Heparesc (even in the context of a conditional or exceptional circumstances MA).

Ground#3. Methodological issues and lack of consistency in the data.

There was no dedicated section in the response document addressing all the points summarised under Ground #3 (post-hoc analyses, sources of bias, missing correlation between the different endpoints used) in a structured and sufficiently detailed manner. The Applicant focused on the issues raised with regard to selection of the control group and the results of the bootstrap analyses.

The Applicant defends the exclusion of patients from the control as being based on a sound rationale and reiterating former arguments. They point out the high severity of UCD in the study population enrolled in CCD02/CCD05 as evidenced by the high rate of OLT of 75% (vs 37% in patients from the RC set and 17% overall in CCD10). Other potential reasons for this discrepancy such as historical vs. recent practice, logistics, regional differences, etc. are not addressed. Exclusion of mild cases with stable disease from the control group is seen as justified in order to define a group with severity of UCD comparable to the treated patients, and this is viewed as being supported by the results of the ureagenesis assay in study CCD09: The CCD02/CCD05 study population had the lowest ureagenesis capacity (close to zero) of all groups measured, while the CCD09 neonatal onset group resembling the mild/stable patients had significantly higher values. Exclusion of early events from the control group is also seen as appropriate as these could be regarded as part of the initial crisis; moreover this was largely resolved by matching according to age at start of treatment as all events earlier than 28 days were omitted.

There have been extensive discussions around the definition of the control group in the previous rounds of assessment. The difficulties of finding/ defining a comparable control group in such a rare disease are well known. The Applicant tried several ways to address the concerns of potential bias due to exclusion of patients by providing several sensitivity analyses, a bootstrap analysis and redefinition of the patient sets by an expert panel.

<u>Ad) definition of the control group, potential bias</u>

While some of the arguments for excluding patients from the control group (post hoc) are plausible it must still be recognized that in a very small sample the exclusion of even a single patient may have impact on the results and must be seen very critically. On the other hand, definition of a control group assumed to match the treated group as closely as possible (as requested by the former Rapporteurs and performed by the Applicant's expert panel) increases the internal validity of the comparison, and UCD subtype and age at start of treatment are also plausible criteria. Nevertheless, it is a post hoc selection and as some of the experts involved in the definition/ selection process were also the clinical trial investigators selection bias is clearly of concern.

<u>Ad) bootstrapping analysis</u>

The chosen approach of bootstrapping analysis is of interest, though a critical discussion of its performance in view of the small sample size is lacking. The opinion of the former Rapporteurs as regards

the statistical analysis is endorsed. These analyses while indicating a trend in favour of the treated group with regard to time to/ incidence of moderate and severe hyperammonaemic events cannot be considered sufficiently convincing as the results generated remain on a very low evidence level. In addition there is still some concern that definition and selection of patient sets might have been biased and to some degree data driven. Nonetheless, the comparative analysis was conducted to provide supportive evidence for the efficacy of Heparesc; it was not the primary analysis.

In conclusion, the CAT was of the view that the major objections identified in the original assessment have not been resolved. While acknowledging the challenges in the clinical development are (very rare disease, vulnerable patient population and complexity of treatment), the CAT is of the opinion that there are still unresolved major concerns regarding the ureagenesis assay and data gathered, methodological issues and lack of consistency in data, and efficacy has not been demonstrated with reasonable certainty; thus the Benefit-Risk balance remains unfavourable.

At the oral explanation to the CAT the applicant requested consideration for MA under exceptional circumstances and proposed a revised indication:

“Treatment of paediatric patients from birth to less than 3 years of age with a severe urea cycle disease, due to a known severe mutation verified by genetic testing or previous death reported with that mutation or uncontrolled hyperammonaemia, whom are considered for a solid organ transplant.

Underlying urea cycle defects that qualify: carbamoylphosphate synthetase 1 deficiency, ornithine transcarbamylase deficiency, argininosuccinate synthetase deficiency”.

The CAT considered the applicant’s proposal for the restricted indication and the claim for exceptional circumstances, but concluded that despite restricting the indication, the unresolved major concerns detailed above still apply. Besides the criteria for approval under exceptional circumstances were not met as the Benefit-Risk of the product remains negative, and therefore the CAT recommended Refusal of the granting of the Marketing Authorisation.

During the OE at the CHMP, the Applicant proposed further restriction to the indication (removing carbamoylphosphate synthetase 1 deficiency from the indication presented at the CAT). The CHMP considered the new proposal and concluded that despite restricting the indication, the unresolved major concerns detailed above still apply.

6. Recommendations following re-examination

Based on the arguments of the applicant and all the supporting data on quality, safety and efficacy, the CAT re-examined its initial opinion and in its final opinion concluded by consensus that

Whereas the efficacy of Heparesc in the treatment of severe urea cycle disorders has not been sufficiently demonstrated:

- Clinical relevance of the observed changes in 13C ureagenesis assay has not been demonstrated and there are still uncertainties regarding the variability of the data gathered from this assay.
- Efficacy has not been demonstrated with reasonable certainty (even in the context of conditional or exceptional circumstance MAA, with limited data), and it is possible that observed trends as regards reduction of hyperammonaemic events are chance findings based on a comparison to historical control with too many biases
- Overall there were many methodological issues in the studies conducted and a lack of consistency in the data presented. Data were analysed post-hoc, and many sources of bias were present. In view of the results presented a strong endpoint was missing, and correlation between the different endpoints used was not demonstrated,

the CAT is of the opinion that pursuant to Article 12 of Regulation (EC) No 726/2004, the efficacy of the above mentioned product is not properly or sufficiently demonstrated.

For these reasons, the benefit/risk balance of Heparesc in the proposed indication is unfavourable and the CAT has recommended the refusal of the granting of the marketing authorisation for Heparesc.

Therefore, based on the arguments of the applicant and all the supporting data on quality, safety and efficacy and the draft opinion adopted by the CAT, and also considering the applicant`s proposal for a further restricted indication and the claim for exceptional circumstances, the CHMP re-examined its initial opinion, and in its final opinion concluded by majority that the benefit/risk balance of Heparesc in the proposed indication is unfavourable.

Therefore, the CHMP has recommended the refusal of the granting of the marketing authorisation for Heparesc.

Divergent position to the majority recommendation is appended to this report.

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APPENDIX 1

Divergent Position

DIVERGENT POSITION

The undersigned members of the CHMP did not agree with the CHMP's negative opinion for Heparesc.

From the data presented by the Applicant a trend in beneficial outcomes can be observed after treatment with Heparesc suggesting extended phases of metabolic stability without hyperammonaemic crises in a number of patients. Comparative analyses versus a historical control group suggest a benefit of Heparesc in preventing/delaying further moderate and severe hyperammonaemic events, even under the most conservative assumptions for definition of the control group. Proof of concept for functionality of the infused liver cells was shown by measurement of ureagenesis capacity in a patient with a genetic null mutation and increases in ureagenesis could also be observed in some other patients. It is, however, acknowledged that precise quantification of the functional improvement is not possible on the basis of these data. Finally, the presence of DNA from the infused liver cells in Heparesc was detected in the explanted livers of the majority of patients who underwent orthotopic liver transplantation at a later stage.

Although it is agreed that according to normal standards of regulatory approval the efficacy of Heparesc in the treatment of severe forms of neonatal onset UCD has not been sufficiently substantiated, the conclusion on benefit/risk has to be taken under consideration of the exceptional circumstances encompassing the clinical condition investigated. The subpopulation of patients presenting with the most severe form of neonatal onset UCD are an extremely rare cohort of patients in an already rare disease. Clinical management of these patients is very challenging and there is an acute need for further therapeutic options in order to enable them to reach a mature enough state for undergoing orthotopic liver transplantation while also preventing (further) neurological deterioration. In such a situation it is not considered possible to conduct a standard controlled clinical trial and it is evident that – in line with the prerequisites as foreseen for an approval under exceptional circumstances – a full clinical data package cannot be obtained.

Taking into account that the risk profile of Heparesc in view of the severity of the condition and the limitation of treatment to specialised centres appears acceptable and manageable, the observed beneficial trends are considered to outweigh the risks and therefore the benefit/risk balance is seen as positive for an approval under exceptional circumstances, pursuant to Article 14 (8) of regulation (EC) No 726/2004.

London, 22 October 2015

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