2 SYNOPSIS

NAME OF COMPANY: Promethera Biosciences	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
NAME OF STUDY DRUG: Human Heterologous Liver Cells for Infusion (HHLivC)		

PROTOCOL NO.: CCD05

TITLE:

Open, Prospective, Historic-Controlled, Multicenter Study to Evaluate the Safety and Efficacy of Infusion of Liver Cell Suspension (HHLivC) in Children With Urea Cycle Disorders**.**

INVESTIGATORS AND STUDY CENTERS: 14 Investigators throughout the United States (n=13) and Canada (n=1).

Center	Investigator Name	Center	Investigator Name
Alberta Children's Hospital	Aneal Khan, MD, MSc	Children's Hospital of Wisconsin	David P. Dimmock, MD
Children's National Medical Center	Uta Lichter-Konecki, MD	Mount Sinai Medical Center	George A. Diaz, MD, PhD
MedStar Georgetown University Hospital	Thomas Fishbein, MD	Oregon Health Center & Science University	Cary O. Harding, MD
Children's Healthcare of Atlanta	Miriam Vos, MD, MSPH	Ronald Reagan UCLA Medical Center	Gerald Stuart Lipshutz, MD
Lucille Packard Children's Hospital	Clark Andrew Bonham, MD	UCSD/Rady Children's Hospital San Diego	Bruce A. Barshop, MD, PhD
Ann & Robert H. Lurie Children's Hospital of Chicago	Barbara K. Burton, MD	University of Minnesota Medical Center	Susan A. Berry, MD
Seattle Children's Hospital	J. Lawrence Merritt II, MD	Yale-New Haven Hospital	Sukru H. Emre, MD, FACS

PUBLICATION(S):

None

PHASE OF DEVELOPMENT: Phase II

PRIMARY OBJECTIVE:

To investigate the safety and efficacy of multiple HHLivC infusions in children with ornithine transcarbamylase deficiency (OTCD), carbamoylphosphate synthetase I deficiency (CPS1D), or argininosuccinate synthetase deficiency (ASSD).

PRIMARY EFFICACY VARIABLE

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) after first HHLivC infusion.

SECONDARY OBJECTIVE:

To investigate the safety and efficacy of multiple HHLivC infusions in children with OTCD, CPS1D, or ASSD as determined by the assessments noted within the secondary variables.

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SECONDARY EFFICACY VARIABLES

- Number, duration and severity of metabolic crises (maximum ammonia concentration, duration of coma)
- Growth, protein intake and nutritional status
- Use of ammonia scavenging drugs
- 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
- Evaluation of the ^{13}C urea formation in the course of the study

SAFETY VARIABLES (for primary and secondary objective)

- Serious adverse events (SAEs): portal thrombosis, systemic shunting of liver cells, other SAEs
- Adverse events (AEs)
- Clinical laboratory parameters to monitor the safety of the procedures and immunosuppression

METHODOLOGY:

This was an open, prospective, multicenter study in children with complete OTCD, CPS1D, or ASSD with neonatal-onset type as confirmed by liver biopsy or DNA analysis who were treated with multiple infusions of HHLivC. Patients were receiving immunosuppression to avoid rejection of allogenic cells.

The study consisted of an enrollment and diagnostic phase (5 days), 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 (FU) phase (until 24 months after the first HHLivC infusion or until 3 months after OLT). Subjects received up to 6 HHLivC infusions over 6 days, each separated by approximately 24 hours during the application phase.

The enrollment of the first 3 subjects under study medication was staggered. One subject was treated at a time with 3 weeks observation period after the last hepatocyte infusion and evaluation of safety through the data monitoring committee (DMC), prior to proceeding to the subsequent subject.

In treated subjects who underwent OLT prior to Visit 19, the 4-month measurement of ^{13}C urea production, was allowed to be scheduled earlier but only in case that Visit 17 (2-month measurement) was conducted at least 4 weeks prior to the new planned Visit 19. The explanted livers of treated subjects who underwent OLT were evaluated for donor cell material. After undergoing OLT, subjects were followed for safety for 3 months post-OLT. Subjects who did not undergo OLT were followed for efficacy and safety until the end of follow up at 24 months.

Data from historical controlled subjects who were treated with current standard of care were planned to be used as controls in interim and final analyses for this study. However, per Amendment 5 (protocol version 1.8), this analysis was removed and the historical controls will be included in an integrated analysis across multiple studies.

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NUMBER OF SUBJECTS (Planned and Analyzed): 21 treatment subjects plus 20 controls were planned. A total of 90 subjects were considered for this study for the North American region. The diagnoses were split as follows: ASSD (n=24), OTCD (n=43), CPS1D (n=9), other indications/not known (n=14). From those 90 subjects, a total of 10 subjects were enrolled into CCD05 and are included in this clinical study report.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION AND EXCLUSION:

Inclusion Criteria:

A subject could have been included in the study if all of the following criteria were met:

1. Males or females whose gestational corrected age (calculated from term delivery or 37 weeks of gestation) on the day of enrollment is 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.
3. Further biochemical parameters and DNA analyses confirmed 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 uM/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 N-acetylglutamate synthetase deficiency (NAGSD) is required to differentiate from a suspected CPS1D diagnosis. Deoxyribonucleic acid (DNA) analysis for pathogenic mutation of CPS-1 is planned after enrollment.

4. Plasma ammonia level ≤250 µmol/L at time of enrollment.
5. Written informed consent obtained from the subject's legal representatives.

Exclusion Criteria:

A subject was not included in the study if any one of the following criteria were met:

1. Weight ≤3.5 kg.
2. Presence of acute infection at the time of inclusion.
3. Severe chronic or systemic disease other than study indication.
4. Structural liver disease (eg, cirrhosis, portal hypertension) or venoocclusive diseases.
5. Portal vein thrombosis.
6. Known diagnosis of hereditary thrombophilia (eg, factor V Leiden, prothrombin 20210A variant) or parental history of hereditary thrombophilia and absence of thrombophilia testing in subject.

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7. 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.
8. Contraindications for immunosuppression.
9. Live vaccination planned during the study.
10. Live vaccination within 4 weeks prior to beginning study.
11. Required valproate therapy.
12. Participation in other clinical trials, or received experimental medication within the last 30 days.

Note: An individual who was initially excluded from study participation based on ≥1 of the above time-limited criteria (eg, acute infection) could have been reconsidered for enrollment once the condition was resolved contingent on the subject continuing to meet all other entry criteria.

TEST PRODUCT, DOSE, MODE OF ADMINISTRATION, AND BATCH NUMBER:

Study Drug: Human Heterologous Liver Cells for Infusion

Cell Dosage, based on children's weight (total cumulative dose):

≤10 kg: 0.3×10^9 viable liver cells per kilogram of body weight
>10 to 15 kg: 3.0×10^9 viable cells nonadjusted to body weight
>15 kg: 0.2×10^9 viable liver cells per kilogram of body weight

Route of Administration

Infusion into the portal vein. A Hickman/Broviac catheter was placed into the portal vein via branches of the inferior or superior mesenteric vein. If the ductus venosus was still open, it would have been closed prior to HHLivC infusion.

Conditions of HHLivC infusion

- Number of individual sessions per subject: 6
- Duration of application phase: 6 days
- Planned time span between sessions: ~ 24 hours
- Number of viable cells per individual session (by body weight):
  ≤10 kg: 0.05×10^9 per kilogram body weight
  >10 to 15 kg: 0.5×10^9 nonadjusted to body weight
  >15 kg: 0.033×10^9 per kilogram body weight
- Number of total cells per individual session: based on a minimum of 50% viable cells in the preparation, the maximum of total cells infused per individual session was (by body weight):
  ≤10 kg: 0.10×10^9 per kilogram body weight
  >10 to 15 kg: 1.0×10^9 nonadjusted to body weight
  >15 kg: 0.066×10^9 per kilogram body weight

Batch Numbers: A list of batch numbers administered per subject is provided in Appendix 16.1.6.

DURATION OF TREATMENT:

Administration of the investigational product started 1 day after the placement of the application catheter. The active treatment was administered over an application phase of up to 6 days, with an observation phase through 6 months after the first HHLivC infusion or until OLT.

STATISTICAL METHODS:

Descriptive statistics were used to summarize currently available subject data, no data from historic controlled

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subjects were considered. Sample size and power considerations were not applicable for this report,

Due to the small number of subjects, all summaries were performed using data pooled across centers. No hypothesis tests were performed. Continuous variables were summarized by presenting the number of non-missing observations, mean, standard deviation, median, minimum, and maximum. Categorical variables were summarized by presenting the number of subjects and percentage for each category. The following 2 analysis sets were included:

Safety Set: This set comprises all subjects who received at least a measurement for placing an application catheter for HHLivC infusion. This set was used for all safety analyses. Safety analysis includes extent of exposure, AEs, SAEs, deaths, laboratory parameters, vital signs, physical examination and nutritional status, catheter placement, and also oxygen saturation, portal blood pressure, and portal vein flow (PVF) during HHLivC infusion.

Efficacy Set (Intent to Treat): This set includes all subjects in the safety set for whom any efficacy assessment was available. In this set, all efficacy endpoints were evaluated. There were no consideration of prognostic factors and no subgroup analysis. Efficacy analysis includes ^{13}C urea concentration (maximum concentration [C_{max}], time to peak concentration [T_{max}], area under the curve [$AUC_{(0\text{-}120min)}$], and $AUC_{(0\text{-latest time point})}$), frequency and severity of metabolic crisis, and listing of laboratory parameters.

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SUMMARY-CONCLUSIONS

The overall median subject age was 120.5 days at enrollment with a range from 23 days to 5 years. All subjects had genetically documented urea cycle disorder (UCD) with 8 subjects diagnosed upon first manifestation after birth. All but 1 subject had a known history of at least 1 hyperammonemic event prior to enrollment. All subjects received ammonia-scavenging agents, steroids and tacrolimus during the study. Mean tacrolimus trough levels ranged from approximately 6 to 15 ng/mL over the first 4 weeks of the study. The mean (SD) daily dose of prednisone during the first 30 days of the study was 6.72 (3.80) mg.

Safety Results:

Catheter placement: Catheterization for HHLivC infusion was successful in all subjects. One subject (26-12-02) experienced laceration of the inferior mesenteric vein during catheter placement and required surgical repair. Subject 26-13-01 was enrolled into the study but did not receive an infusion of HHLivC. The subject underwent exploratory laparotomy and insertion of a Broviac catheter. Due to persistent high portal vein pressure (PVP) ranging from 19-20 mmHg, the subject was terminated from the study. The catheter was subsequently removed and the increased PVP was considered to have resolved.

Administration of HHLivC: Seven of the 9 subjects received all 6 infusions of HHLivC. One subject experienced device dislocation after the 5th infusion and 1 subject developed bilateral femoral deep vein thrombosis secondary to the central venous catheter on study Day 4. Portal vein pressure, O_2 saturation and vital signs generally remained unchanged pre and post infusions. None of the subjects experienced any complications with catheter removal.

Follow-up: The most frequently occurring events were hyperammonemia, vomiting, anemia and hypokalemia. The most frequent (occurring in 2 or more subjects) AEs considered related to HHLivC were vomiting, rash, hypotension, tachycardia, portal vein thrombosis and rash erythematous. Two subjects were withdrawn due to AEs. In addition to the subject who developed bilateral femoral deep vein thrombosis, one subject's condition significantly deteriorated and was withdrawn from the study for palliative care.

One subject (15-15-03) died during the study due to complications of UCD and one subject (11-11-02) died shortly after withdrawing from the study due to unknown causes. Both deaths were considered unrelated to HHLivC or study procedures.

None of the subjects showed signs of malnutrition (i.e., skin or hair) on scheduled visit days and there was a trend toward increased mean and median head circumference, body length and weight from pre-study to Month 6.

Efficacy Results:

For subjects with ^{13}C measurements, an increase in mean AUC and C_{max} and decrease in T_{max} was observed at Month 2 and Month 4, however there was substantial variability in the results across individual subjects. Since a spontaneous improvement of the urea cycle activity over time is not expected nor do concomitant therapies exert any effects on the intrinsic activity of the urea cycle, it is concluded that the observed increase in ^{13}C-urea formation can only be attributed to a sustained engraftment of functional donor hepatocytes into the subjects' livers.

The results from this small number of subjects have been included in a separate integrated analysis of the experience of subjects across multiple studies.

Conclusions:

This study was performed to test the safety and efficacy of HHLivC infusions in children with urea cycle disorders. Overall conclusions from this study are the following:

- Placement and subsequent removal of the catheter in these subjects was successful and can be effectively managed in specialized centers.

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- Infusion of HHLivC at the target number of cells was well tolerated in these subjects.
- There was a trend in an increase in mean AUC and C_{max} in subjects who had evaluable ^{13}C measurements. These data will be combined with data from other studies for additional analyses.
- HHLivC infusions were well tolerated in this highly vulnerable and co-morbid patient population.