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Clinical trial implementation for congenital urea cycle disorder, using human ES cells

— The world's first successful transplantation of human ES cell-derived hepatocytes into humans —

Tokyo, Japan, May 18, 2020 — The National Center for Child Health and Development (NCCHD)'s group consisting of Mureo Kasahara, Executive Director, Akinari Fukuda, Medical Director, and Seisuke Sakamoto, Deputy Director, of the Organ Transplantation Center, Reiko Horikawa, Medical Director of Endocrinology and Metabolism, and Akihiro Umezawa, Director of the Center for Regenerative Medicine, conducted the world's first doctor-initiated clinical trial entitled, "Exploratory study into neonatal patients/Clinical trial of HAES transplantation therapy for congenital urea cycle disorder" on October 21, 2019. This was a clinical trial conducted on humans using human ES cell²⁾ -derived hepatocytes (HAES) produced at the National Center for Child Health and Development¹⁾ and the success of the treatment was an unprecedented achievement in the world.

The patient in this clinical trial was a 6-day-old baby suffering from urea cycle disorder, preventing it from decomposing toxic ammonia. This clinical trial was a hepatocyte transplantation in which hepatocytes derived from human embryonic stem cells (ES cells) were injected into the blood vessels of the liver (Fig. 1) and was performed as a "bridge treatment" until the patient was able to undergo a liver transplant safely, at the age of 3 to 5 months after birth (weighing approximately 6 kg). Following the hepatocyte transplantation, the patient did not see an increase in blood ammonia concentration and was able to successfully complete the next treatment, a liver transplantation.

Although the biggest problem in conventional hepatocyte transplantation therapy was that a stable supply of hepatocytes could not be obtained, hepatocytes were stably produced this time from ES cells. It is





anticipated, from the success of this trial, "the bridge treatment", that newborns who were unable to undergo liver transplantation will be safely able to do so.



[Fig. 1 Flow of human ES cell-derived hepatocyte transplantation in this trial]

[Points of the press release]

- The first clinical trial in humans using human ES cells in Japan. It is also the world's first clinical trial to use human ES cell-derived hepatocytes for the treatment of liver diseases.
- In this clinical trial, NCCHD was able to verify the safety and efficacy of transplanting hepatocytes prepared from human ES cells into humans (there were no complications associated with the surgical procedure).
- In this clinical trial, hepatocyte transplantation was completed on the first subject in the world. NCCHD was able to safely introduce patients, who have not been able to undergo a liver transplant until now, to liver transplantation.
- This treatment method is expected to be an effective addition to the conventional treatment method for avoiding hyperammonemia attacks, during the waiting period until liver transplantation, which is the curative treatment method for patients with





congenital urea cycle disorders, along with their families.

This treatment method could be benefit in the future from potential success in cell/ gene treatment.

[Background and Objective]

Liver transplantations for newborns are not only technically difficult, but also often lead to serious life-threatening complications such as respiratory failure and impaired blood flow due to abdominal pressure because the transplanted liver is too large relative to the abdominal volume of the patient. Therefore, liver transplants are usually performed after waiting for the patient to reach a safe weight of 6 kg.

"Urea cycle disorder³⁾⁻⁶⁾," which is the target disease of this clinical trial, is a congenital disease that develops shortly after birth; as a consequence thereof, patients have to wait 3 to 5 months until they grow to a size that allows liver transplantation (6kg in weight), while undergoing restricted protein intake⁷⁾, drug therapy, and hemodialysis treatment⁸⁾. In Europe and the United States, where they can use hepatocytes that are isolated and cryopreserved from livers removed from brain-dead donors, treatments have been performed to decrease in blood ammonia levels by transplanting hepatocytes in the neonatal period immediately after onset in order to make this waiting period safer. However, in Japan, where hepatocytes from brain-dead liver transplant donors are rarely available, a stable supply of stable quality hepatocytes as the cell source for hepatocyte transplantation has been the greatest challenge for this disease.

Therefore, the center has focused on hepatocytes derived from pluripotent stem cells (human ES cells), which can be proliferated almost indefinitely as a new cell source. By storing human ES cells in a state in which they are induced to differentiate into hepatocytes and cryopreserved, they can be used for emergency transplantations as well. The center has been recognized as one of the only two ES cell establishment institutions in Japan aiming to establish a new treatment method that can safely introduce newborns to liver transplantations using human ES cells.





[Patient's symptoms and clinical trial outline/results]

\leq 2nd day of life \geq

Although the patient in this trial had no problems at birth and 24 hours after birth, symptoms such as polypnea, hypertonia, and convulsions appeared on the second day after birth. Blood testing revealed a marked hyperammonemia of 2,026 μ g/dl (normal value is 66 μ g/dl or less) and the patient was transferred to NCCHD for suspected urea cycle abnormalities, where continuous hemodiafiltration and drug therapy was initiated. Subsequently, biochemical and genetic tests confirmed the diagnosis of severe urea cycle deficiency (citrullinemia type I).

<6th day of life>

Upon obtaining consent for HAES hepatocyte transplantation, eligibility of subject candidates by the efficacy and safety evaluation committee, and approval for conducting clinical trials, 190 million cells of HAES hepatocyte were administered over 2 days while monitoring the portal pressure and portal blood flow. The administration was completed without complications or adverse events due to the hepatocyte transplantation procedure.



[Fig. 2 Actual state of hepatocyte transplantation] A thin tube called a catheter was inserted from the umbilical vein (umbilical cord blood vessel) and the tip was placed in the portal vein in the liver, after which HAES (human ES cell-derived regenerative medicine product, upper-left, upper-right, lower-left) was slowly injected little by little via syringe(lower-right).





< 9 days following hepatocyte transplantation >

The general condition was good and the patient left the ICU to be moved to general ward management.

< 9 weeks following hepatocyte transplantation >

The patient was discharged from the hospital because the patient's blood ammonia level did not rise after gradually increasing the protein intake and the patient's body weight increased to about 5 kg.

\leq 3 months following hepatocyte transplantation \geq

A blood test at the time of regular outpatient visits indicated no abnormalities of note, including ammonia levels.

<5 months following hepatocyte transplantation>

Performed living donor liver transplantation with the father as a donor. After the immunosuppressive therapy was intensified for the rejection, the condition of the patient improved and subsequently passed without complications.

<2 months following living liver transplantation>

Left the hospital (without any surgical complications and neurological sequelae). 6 months after birth at discharge.

[Selection criteria/Exclusion criteria for this clinical trial]

A child patient with neonatal-onset congenital urea cycle disorder, which is the selection criterion, with low body weight (6 kg or less) and scheduled to undergo liver transplantation when it is determined safe to undergo liver transplantation. Furthermore, diseases such as heart disease, lung disease, neurological disease, malignant tumor, etc., which are suspected to be infected with hepatitis B virus, hepatitis C virus, AIDS virus, which are the exclusion criteria, must be ruled out.

[Future outlook/Comments from presenters]

• The success of this trial demonstrates safety in the world's first clinical trial using human ES cells for patients with liver disease. It is expected that this clinical trial will be used as a model case, leading to the development of regenerative medicine products related to liver diseases.

• Going forward, NCCHD will continue to carry out cases and continue to verify the safety and efficacy.⁹⁾





• In Japan, the establishment of treatment methods for more intractable diseases and improved treatment results are expected, by making the most of the advantages of ES cell-derived products for various organs and diseases.

[Remarks]

This research and development was carried out with cooperation as part of the Regenerative Medicine Realization Network Program and the Regenerative Medicine Practical Research Project, of the Japan Agency for Medical Research and Development (AMED).

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About the National Center for Child Health and Development

The National Center for Child Health and Development (NCCHD) was established in 2002 by combining the National Okura Hospital and the National Children's Hospital. The NCCHD's philosophy emphasizes cooperation between its hospital and research wings to promote medical care and research aimed at fostering healthy future generations. The NCCHD is the largest hospital in Japan specializing in pediatrics, perinatal care, obstetrics, and maternal medicine and has 490 beds and an average of about 1000 daily outpatient visits. The NCCHD provides comprehensive and continuous "developmental care" encompassing every stage of life, from the fetus, neonate, infant, toddler, school-aged child, and adolescent to the adult, as well as future generations.

In addition, the center conducts research on elucidating the etiology and pathology of diseases and finding cures while also offering insights on building a society for healthy future generations.





[References]

<1: Research Institute National Center for Child Health and Development>

The Research Institute National Center for Child Health and Development (Director: Yoichi Matsubara) is one of the only two institutions of human ES cell establishment in Japan, acting as the base for human ES cell research in Japan. The National Center for Child Health and Development has so far established 7 human ES cells (SEES1-7). Akihiro Umezawa from the Center for Regenerative Medicine, as the core person, and others are developing HAES (human ES cell derived hepatocyte-like cells) for hepatocyte transplantation therapy for patients with urea cycle disorders using human ES cells (Fig. 3, 4). As the only institute in Japan, which conducts clinical trials of products such as regenerative medicine made from ES cells in compliance with the pharmaceuticals and medical devices law.



[Fig. 3 Preparation of HAES (human ES cell-derived regenerative medicine products)]







[Fig. 4 HAES (human ES cell-derived regenerative medicine products) cryopreserved in a liquid nitrogen container **]**

\leq 2: What are human ES cells?>

ES cell is an abbreviation for "Embryonic Stem Cell," which is a stem cell made using the inner cell mass of the embryo. Because human ES cells are made from the cells of human embryos in early development, they have the ability to transform into any cells that make up our body, similar to fertilized eggs. ES cells are said to be able to semi-permanently maintain the proliferative and pluripotent properties thereof, as long as they are maintained in an appropriate culture environment. It is also known that when a culture environment that maintains pluripotency is transferred to a culture environment that differentiates into nerves or blood, it will differentiate into various cells depending on the environment.

< 3: What is a congenital urea cycle disorder?>

The "urea cycle" is a pathway that converts toxic ammonia (NH3), generated in vivo, into nontoxic urea, mainly in the liver (Fig. 5). Urea cycle disorders are a group of diseases that have congenital abnormalities in the metabolic system of the urea synthesis pathway and develop due to symptoms such as hyperammonemia.







[Fig. 5 Enzyme deficiency site in urea cycle disorder (citrullineemia type I)] The urea cycle is a metabolic pathway that takes in blood ammonia (a toxic substance) into the hepatocytes and converts it into harmless urea. There are enzymes that promotes metabolism at each stage, so diseases are diagnosed depending on which enzyme is missing. In this case, because the argininosuccinate synthase that promotes the synthesis of argininosuccinate from citrulline is congenitally deficient, the urea cycle stopped, while hypercitrullineemia developed, causing severe hyperammonemia.

< 4: Causes of congenital urea cycle disorders >

Because this is an inherited illness, it leads to an increased probability of developing urea cycle disorders if a family member has a urea cycle disorder. N-acetylglutamate synthase deficiency, carbamylphosphate synthase deficiency, citrullinemia type I, argininosuccinic aciduria, and argininemia, are inherited in the hereditary form of "autosomal latent inheritance," while ornithine transcarbamylase deficiency is in the form of "X-linked inheritance." With autosomal latent inheritance, parents are carriers and their children have a 1/4 chance of developing it. Carriers do not exhibit any symptoms at all and no treatment is required. However, the onset of mutations is not uncommon.

\leq 5: Symptoms of congenital urea cycle disorder \geq

Symptoms include vomiting, poor suckling, polypnea, convulsions, consciousness disorders, behavioral abnormalities, developmental disorders, etc. and are sometimes potentially serious and life threatening.





< 6: Number of patients with congenital urea cycle disorder >

The incidence of urea cycle disorders is estimated to be 1: 8,000 to 44,000.

Disease name	Frequency	Estimated number
		of domestic patients
Carbamyl phosphate synthase deficiency	1 in 800,000	Approx. 100
Ornithine transcarbamylase deficiency	1 in 80,000	Approx. 500
Citrullinemia type I	1 in 530,000	Less than 100
Argininosuccinic aciduria	1 in 70,000	Approx. 100
Argininemia	1 in 2.2 million	Less than 100

[Table 1 Patient incidence and estimated number of patients by disease in Japan]

<7: Treatment of congenital urea cycle disorders>

Dietotherapy and drug/amino acid therapy are the basic therapies. Intravenous feeding, central venous nutrition, and hemodialysis may be performed in the acute phase. Although dietotherapies can limit protein intake, they must be properly followed to prevent malnutrition. Sodium benzoate and sodium phenylbutyrate are drugs that excrete ammonia out of the body. Amino acid therapy includes arginine and citrulline and is used for diseases that lack arginine and citrulline, among urea cycle disorders. For cases of uncontrolled hyperammonemic seizures, liver transplantation is the radical treatment.

 \leq 8: Reasons why liver cell transplantation is necessary for patients with congenital urea cycle abnormalities and liver transplantation difficulty in the neonatal period \geq

Although it is desirable to have a liver transplant as early as possible, liver transplantation for newborns comes with a high risk of serious postoperative complications leading to death; therefore, they have to wait until weighing approximately 6 kg and until it is relatively safe to undergo a liver transplant. However, during the 3 to 5 months waiting for weight gain up to 6 kg, severe ammonemia may be seizure-induced even with the above treatment and the higher the ammonia level and number of seizures, the more severe any damage to the brain may become, resulting in death in many cases. Even if a liver transplant is finally achieved, damage to the brain is irreversible, necessitating a "bridge treatment" until the liver transplant can be safely performed. Hepatocyte transplantation has been expected to be effective, in addition to conventional treatments, as a treatment that can be safely performed even for newborns shortly after birth.







[Fig. 6: The role of hepatocyte transplantation: the need for "bridge treatment" until liver transplantation can be safely performed]

\leq 9: Safety of this clinical trial \geq

In this clinical study, all the administered hepatocytes settled in the portal vein in the liver or in the sinusoids beyond that (the area where hepatocytes are lined up) and did not spread throughout the body. Due to completely removing the autologous liver during liver transplantation, as an organ transplant, it makes it possible to evaluate whether ammonia-metabolizing enzymes were produced, a more objective evaluation of safety becomes available that has not been found in cell transplant treatments other than the liver.





<A Letter from a patient's family>

First of all, we would like to express our heartfelt gratitude on behalf of our child for receiving this treatment and being able to leave the hospital safely and healthily. We would like to thank all the doctors, nurses, hospital staff, researchers, and everyone involved.

Let us tell you about our experience. Soon after we were emotionally moved by the safe birth of our child, some symptoms suddenly appeared, and we were struck with despair. Our child was taken to NCCHD and found to have an intractable disease found in one out of tens of thousands of people.

Thanks to the doctors' hard work, our child managed to survive. However, we were told that a lifethreatening attack could happen again at any time.

The only hope was one particular treatment. The doctor said, "This is the first such treatme nt in the world." We wondered, "Why does our child have to undergo such a difficult thing?". After much deliberation, we decided that it was all right because the doctor w ho saved our child's life told us so, and we went this far with trust.

During the treatment, there were times when we were worried and anxious, and it was not an easy path. As we came to understand how the family of a patient suffering from the same intractable diseases would feel, we would like to tell this experience to them, give advice, and give them the courage to keep going on.

From now on, we will look forward to the growth of our child who was saved. Once again, we would like to thank all of you who have been involved in providing cutting-edge medical care through years of research for this treatment.

Thank you very much.