LCAT-trial-24 weeks: Protocol for a clinical study to evaluate the safety of regenerative medicine and gene therapy by the autologous transplantation of human lecithin:cholesterol acyltransferase gene-transduced human pre-adipocytes

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ABSTRACT

Backgrounds: Despite the absolute need for life-long treatment of inherited and genetic diseases, there has been little effort to develop such treatments for most of these conditions due to their rarity. Familial lecithin: cholesterol acyltransferase (LCAT) deficiency is recognized as one such orphan disease. We have been developing an adipocyte-based ex vivo gene therapy/regenerative medicine, a novel methodology that differs from the adeno-associated virus-mediated in vivo gene therapy or ex vivo gene-transduced hematopoietic cell therapy, to treat familial LCAT deficiency. Recently, a first-in-human (FIH) clinical study was conducted under the Act on Securement of Safety of Regenerative Medicine, wherein a patient with familial LCAT deficiency was treated. To obtain approval to put this treatment into practical use, a clinical trial has been designed with reference to the FIH clinical study.

Methods: An interventional, open-label, unblinded dose-escalation trial was planned, referring to previous FIH clinical study. The trial aims to evaluate the safety of the investigational product in relation to the characteristics of the investigational product (ex vivo gene/cell therapy product by retroviral vector-mediated LCAT gene transduction) using two doses, and the efficacy of the treatment will be evaluated exploratively. A total of three patients will be enrolled sequentially and followed for 24 weeks after administration. This study is designed as a multicenter trial, with Chiba University Hospital administering and evaluating the safety/efficacy of the investigational products at the prescribed visit.

Conclusion: This clinical trial is expected to facilitate the provision of lifelong treatment to many patients with LCAT deficiency.

Trial registration number: Japan Registry of Clinical Trials (jRCT2033200096).

1. Introduction

Enzyme or protein replacement therapy (ERT) is effective as a treatment for plasma enzyme or protein deficiency. Although native or recombinant protein preparations have been administered for these diseases, ERT with such protein preparations has a short duration of action and requires repeated administration, which significantly impairs the quality of life (QOL) of these patients and their families. In contrast,
gene therapy and regenerative medicine, which can continuously produce proteins in the body, are considered suitable for life-long treatment. However, its practical application is limited, and at present, no cure has been developed for most of these diseases.

Familial lecithin:cholesterol acyltransferase (LCAT) deficiency is a rare recessive disorder characterized by low levels of lipoprotein (HDL)-cholesterol and markedly reduced cholesterol ester levels in lipoproteins; it was designated an intractable disease in July 2015 in Japan. Patients often develop severe complications, such as corneal opacity, anemia, and proteinuria, which are suggested to be caused by abnormal lipid deposition. Proteinuria often progresses to renal failure, which leads to the poor prognosis of the patients.

A clinical trial for recombinant LCAT enzyme replacement has been reported in a patient with familial LCAT deficiency [12]. Bolus injection of recombinant LCAT led to peak LCAT plasma concentrations of around 30–40 μg/ml in the patient with the highest dose (9.0 mg/kg), which is 6–8 times as high as in normal subjects. Although plasma lipid levels were normalized and the clinical parameters related to the anemia and renal functions were improved, the effects were transient, as with other conventional ERPs. An immune response reportedly appeared in some of the participants receiving the highest dose of rhLCAT in another clinical trial (NCT02601560) [13]. Therefore, we focused on familial LCAT deficiency for the clinical development of life-long ERT via adipocyte-based ex vivo gene therapy/regenerative medicine products.

During the clinical development, the first-in-human (FIH) clinical study under the Act on Securement of Safety of Regenerative Medicine was conducted (Trial registration number, JRCTa030190230). A patient was registered in January 2017, and LCAT gene-transduced pre-adipocytes were administered; the patient has been followed up until 240 weeks post-administration after the 24-week-long observation period. As a result, an increased blood LCAT activity persisted for 240 weeks (manuscript submitted). Taken together with a previous report of the adipocyte lifespan [8], this result suggested that the effects of this treatment may last for the rest of the patient’s life. No abnormalities were observed according to a tumorigenicity test of the administered cells. RCR was not detected in either any of the cell samples nor in any of the patient’s blood samples for the 240-week-long study period. The only adverse event associated with this treatment was pain at the site of administration, which also resolved rapidly in the FIH clinical study.

To obtain approval to put this approach into practical use, a clinical trial has been designed with reference to that FIH clinical study. The protocol has been created based on consultation with the Pharmaceutical and Medical Devices Agency (PMDA). In April 2020, we submitted a clinical trial notification after approval by the IRB of Chiba University Hospital. After responding to the inquiries in the 30-day survey, the implementation plan, revised based on the inquiries, was approved by the IRB of Chiba University Hospital in June 2020, and the clinical trial was started.

2. Methods and analysis

2.1. Objectives

The purpose of this trial is to evaluate the safety of regenerative medicine/gene therapy by auto-transplantation of LCAT-transduced human pre-adipocytes for 24 weeks after administration in patients with familial LCAT deficiency. The efficacy of the treatment will be evaluated exploratorily.

2.2. Design of the trial

This trial is an interventional, open-label, unblinded dose-escalation trial. This protocol meets the criteria of the Standard Protocol Items: Recommendations for Interventional Trials 2013 statement. A local principal investigator (PI), supported by at least two other staff members (e.g. a research nurse or clinical research coordinator), is conducting the study at each participating site.

This study is designed as a multicenter trial due to the rarity of the disease in Japan, with Chiba University Hospital managing and evaluating the safety/efficacy of the investigational products at the prescribed visit, while medical institutes other than Chiba University Hospital collect information on adverse events (AEs) and concomitant medications/therapies between subject visits. This trial will be conducted for 24 weeks for each patient after administration of the investigational product, followed by a 216-week-long term safety/efficacy trial. Thus, each subject will be evaluated for a total of 240 weeks in those two trials. The outline of the clinical trial is shown in Fig. 1. The schedule of these trials and clinical tests at the prescribed visits is summarized in Tables 1 and 2, respectively.

Since the investigational product is derived from the autologous adipocytes obtained from each patient, and there has so far been no report of excessive disease associated with LCAT protein, the risk of immune/allergic reaction is considered to be low. With reference to the prior first-in-human clinical study conducted under the Act on Securement of Safety of Regenerative Medicine, 1.6 × 10⁷ cells per kg of body...
Blood samples are examined by the exogenous substrate method, endogenous substrate method, and the high-sensitivity exogenous substrate method (using

Blood sample for the RCR test can be taken on Day 1 after administration.

Allowance

MRI examination is conducted in addition to observation at the surgical site.

If there is no safety problem in the first case, the dose will be proceeded to the second case with a dose of 3.2 \( \times \) 10^7 cells/kg body weight.

The Data Monitoring Committee will evaluate the safety of the second dose based on the results at 12 weeks after administration in the second case and will discuss whether or not to enroll a third case with the PI and Study Coordination Committee. If the safety in the second case is confirmed with reference to the opinion of the Data Monitoring Committee, the PI and sub-investigators will proceed with treating the third case using the same dose as in the second case (Fig. 2, Design 1). Based on the opinion of the Data Monitoring Committee, if the doctors of Plastic and Reconstructive Surgery and the Clinical Trial Coordination Committee decide that there are evident safety issues, such as irritation and/or strong pain at the administration site, or systemic findings, such as a fever, not seen in the first case, the dose for the third case shall be the same as for the first case (Fig. 2, Design 1). Based on the opinion of the Data Monitoring Committee, if the doctors of Plastic and Reconstructive Surgery and the Clinical Trial Coordination Committee decide that there are evident safety issues, such as irritation and/or strong pain at the administration site, or systemic findings, such as a fever, not seen in the first case, the dose for the third case shall be the same as for the first case (Fig. 2, Design 1).

In addition, if an AE or defect for which a causal relationship with the investigational product cannot be ruled out occurs during the clinical trial period, the decision on whether or not to continue the clinical trial will be made promptly based on the opinion of the Data Monitoring Committee.

The dose for application of pharmaceutical approval of the LCAT gene-transduced human pre-adipocytes will be determined based on the comprehensive judgment of safety and efficacy in the three cases in this

Table 1

| Screening test | Clinical trial product manufacturing period | Hospitalization after administration |
|----------------|--------------------------------------------|-------------------------------------|
| Allowance (day) | Liposuction | Observation after liposuction | The day before administration/administration | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 |
| Day-42 to -28 | 0 | 0 | ±3 | -2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Medical examination | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infectious disease test | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Observation of liposuction site | ±3 | ±3 | ±3 | ±3 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 |
| Observation at the administration site | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 |
| Hematological examination | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Blood biochemical test | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Urolysis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pregnancy test | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RCR/malignant tumor test | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anti-LCAT, anti-FBS antibody test*5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Blood LCAT activity*5, LCAT concentration | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ophthalmologic observation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Adverse event information/combo drug/combo therapy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Observation period 1 (every 2 weeks) | Observation period 2 (every 4 weeks) | At the time of cancellation | Long-term safety/efficacy clinical trial [240 weeks after administration] (every 24 weeks) |
|----------------------------------------|----------------------------------------|--------------------------|----------------------------------------|
| W | W | W | W | W | W | W | W | W | W | W | W | W |
| 2 | 4 | 6 | 8 | 10 | 12 | 16 | 20 | 24 | 36 | 72 | 120 | 168 | 216 | 48 | 96 | 144 | 192 | 240 |

| Allowance (day) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Medical examination | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infectious disease test | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Observation of liposuction site | ±3 | ±3 | ±3 | ±3 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 |
| Observation at the administration site | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 |
| Hematological examination | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Blood biochemical test | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Urolysis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pregnancy test | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RCR/malignant tumor test | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anti-LCAT, anti-FBS antibody test*5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Blood LCAT activity*5, LCAT concentration | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ophthalmologic observation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Adverse event information/combo drug/combo therapy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*1: Administration of the investigational product (Day 1) is conducted between 21 and 24 days after liposuction.

*2: MRI examination is conducted in addition to observation at the surgical site.

*3: Allowance – 4 days.

*4: Blood sample for the RCR test can be taken on Day 1 after administration.

*5: Blood samples are examined by ELISA.

*6: Blood samples are examined by the exogenous substrate method, endogenous substrate method, and the high-sensitivity exogenous substrate method (using 3H-cholesterol).

weight are to be administered, and the safety will be observed in the first case. If there is no safety problem in the first case, the dose will be doubled (3.2 \( \times \) 10^7 cells/kg body weight) in the second and third cases.

The Data Monitoring Committee will evaluate the safety (and efficacy) of the first dose 12 weeks after administration and discuss with the principal investigator and the Study Coordination Committee whether or not to proceed to the second case and increase the dose. If there are judged to be no safety problems in the first case, based on the opinions of the Data Monitoring Committee, the PI and sub-investigators will proceed to the second case with a dose of 3.2 \( \times \) 10^7 cells/kg body weight. The clinical trial will be discontinued if it is determined that there is a safety problem in the first case. The Data Monitoring Committee will evaluate the safety of the second dose based on the results at 12 weeks after administration in the second case and will discuss whether or not to enroll a third case with the PI and Study Coordination Committee. If the safety in the second case is confirmed with reference to the opinion of the Data Monitoring Committee, the PI and sub-investigators will proceed with treating the third case using the same dose as in the second case (Fig. 2, Design 1). Based on the opinion of the Data Monitoring Committee, if the doctors of Plastic and Reconstructive Surgery and the Clinical Trial Coordination Committee decide that there are evident safety issues, such as irritation and/or strong pain at the administration site, or systemic findings, such as a fever, not seen in the first case, the dose for the third case shall be the same as for the first case (Fig. 2, Design 1). In addition, if an AE or defect for which a causal relationship with the investigational product cannot be ruled out occurs during the clinical trial period, the decision on whether or not to continue the clinical trial will be made promptly based on the opinion of the Data Monitoring Committee.

The dose for application of pharmaceutical approval of the LCAT gene-transduced human pre-adipocytes will be determined based on the comprehensive judgment of safety and efficacy in the three cases in this

The dose for application of pharmaceutical approval of the LCAT gene-transduced human pre-adipocytes will be determined based on the comprehensive judgment of safety and efficacy in the three cases in this.
The primary endpoint of the trial is the occurrence of AEs for which a relationship with the characteristics of the investigational product (ex vivo gene/cell therapy product by retroviral vector-mediated LCAT gene transduction) cannot be ruled out, including:

1. The appearance of replication competent retrovirus (RCR) after the administration of investigational products
2. Tumorigenesis at the injection site after administration of the investigational product (complementarily evaluated by the following three items):
   A. Malignant tumor test (TK activity)
   B. Magnetic resonance imaging
   C. Tumorigenicity test of the investigational product in immunodeficient (NSG) mice
3. The appearance of anti-LCAT antibody after the administration of investigational products
4. Occurrence of unexpected serious AEs other than 1 to 3

The secondary endpoints related to safety are the type, frequency, and severity of AEs other than the primary endpoints that occur after obtaining informed consent, and anti-FBS antibody. The secondary endpoints related to efficacy are changes in blood biochemical test values evaluated during the observation period, including LCAT activity, blood LCAT concentrations, cholesteryl ester/to total cholesterol ratio, HDL-cholesterol, total cholesterol, and low-density lipoprotein (LDL)-cholesterol.

2.4. Inclusion criteria

Patients who meet all of the following conditions are to be included:

1. Patients with a definitive diagnosis of familial LCAT deficiency based on the following diagnostic criteria:
   1) LCAT mutation has been confirmed in a genetic diagnosis (presence of an amino acid residue substitution in LCAT protein)
   2) A low HDL-cholesterol level (<25 mg/dL) is observed, and one or some of the following complications typical for this disease are detected; corneal opacification, renal dysfunction (proteinuria), and hemolytic anemia.
   3) The blood LCAT activity (formally approved in vitro diagnostics or an alternative method using artificially synthesized liposomes) is below the detection limit.
2. Age over 16 years old.
3. Provided their written informed consent. If the patient is a minor, written consent must be obtained from the patient as well as a parent or guardian.

2.5. Exclusion criteria

Patients who meet any of the following conditions are not included.

1. Patients who are not expected to express full-length LCAT protein due to frameshift or the appearance of a stop codon by an insertion/deletion mutation in LCAT.
2. Patients whose mutant LCAT protein cannot be detected in blood by an immunological method (an enzyme-linked immunosorbent assay or immunoprecipitation Western blot).
3. Patients with advanced liver disease (fulminant hepatitis, cirrhosis, etc.) that affects lipid metabolism.
4. Patients with advanced renal disease (transferred or planned to transfer to dialysis).
5. Patients who received LCAT protein replacement therapy via whole-blood or plasma transfusion within one month before written consent was obtained.
6. Patients in whom subcutaneous liposuction is considered difficult.
7. Pregnant and lactating women. For female patients who can become pregnant (all women except those who have undergone permanent contraception or those who are postmenopausal) as
well as male patients, those who do not agree to contraception during the clinical trial period (up to 24 weeks after administration) shall be excluded.

8. Patients with other similar diseases that are not caused by LCAT deficiency and have a low HDL-cholesterol level (Apo A-1 abnormality/Tangier disease).

9. Patients with a history of hypersensitivity to the components of this investigational product or the components (fetal bovine serum and human albumin) used in the manufacturing process of the product.

10. Other patients who are deemed unsuitable by the PI or sub-investigator.

2.6. Treatment methods

Patients who meet all of the inclusion criteria and do not meet any of the exclusion criteria will be registered. Abdominal subcutaneous adipose tissue will be aseptically removed from each patient in the central operating room of the Chiba University Hospital using a liposuction cannula under local anesthesia. The adipose tissue will then be transported to the cell processing center of the Center for Advanced Medicine in the Chiba University Hospital. LCAT gene-transduced pre-adipocytes will be manufactured via a GCTP production procedure by CellGenTech, Inc. (1-8-15, Inohana, Chuo-ku, Chiba-city, Chiba 260-0856 Japan). Propagated cells will be tested to meet quality standards by CellGenTech, Inc. The cells will also be confirmed to demonstrate RCR negativity according to the findings of the NAT-based method.

After the quality tests have been completed, the investigational products will be shipped to Chiba University Hospital and subcutaneously administered to each patient by doctors of Plastic and Reconstructive Surgery under local anesthesia. While there should be no residual LCAT gene-expressing retroviral vector in the investigational products at the time of shipment, subjects will be managed in a private room in order to prevent any potential spread of RCR into the environment. After confirmation of RCR negativity by NAT-based method in the blood sample of the subject, the patient will be released from the private room. If RCR is detected, management in a private room will be continued. If a risk of tumorigenesis of the administered cells or a severe adverse event such as the appearance of RCR occurs during the observation period, then appropriate clinical examinations such as a pathological examination of the administration site should be performed. If necessary, the removal of the administered cells and/or other essential treatments should also be performed.

2.7. Discontinuation of individual cases

If the criteria presented below are met, then the administration will be discontinued, and the PI or sub-investigator will discontinue the study. However, if liposuction to produce the investigational product has already been performed, administration should be discontinued after taking appropriate measures, such as removal of threads placed after fat removal. The discontinuation criteria are as follows:

1. When deviation from the quality standard of the investigational product is found
2. When the investigational product cannot be administered within 24 days after the liposuction
3. When the subject is found to be ineligible for the trial
4. When a tumor requiring treatment is found before administration of the investigational product
5. When there is a request to cancel participation in the trial by either the enrolled subject or the substitute; in such cases, the subject and the PI will have an interview, discuss the method for obtaining safety information as much as possible about the subject’s condition, and take subsequent measures with an agreement.
6. When the PI or sub-investigator determines that the subject cannot continue the clinical trial.

2.8. Data management, monitoring, safety, and auditing

Monitors will ensure that the investigational team is complying with the study protocol and Good Clinical Practice (GCP) standards, that the data and AEs are accurately and appropriately recorded in the electric case report forms (eCRFs), that severe AEs (SAEs) are reported to the

Fig. 2. Design of the LCAT-Trial-24 weeks. In this trial, a total of three patients will be enrolled sequentially. In the first case, the subject will be given the same dose of the investigational product as in the FIH clinical study. The propriety of enrollment and the dose to be administered to the second and third cases will be determined based on the recommendations of the DMC. The dosage for the second and third cases (for the continuation of the trial) will be decided by the principal-investigator based on the recommendations of DMC.
trial coordinator and the investigational drug provider and that those meeting the SAE reporting criteria are reported to the IRB. AEs will be classified in accordance with the Medical Dictionary for Regulatory Activities, Japanese translation MedDRA/J V.22.0 (MedDRA Japanese Maintenance Organization, Tokyo, Japan). All participants with AEs are to be followed up during the course of the AE until their resolution or for four weeks after the end of the trial. All SAEs will be reported to all investigators, discussed through a web-based AE reporting system, and reported to the PMDA, if necessary.

2.9. Data Monitoring Committee

The investigator will establish a Data Monitoring Committee that can monitor the safety of this study and make recommendations on doses of the investigational products. The Data Monitoring Committee shall be independent of the investigators.

The committee will objectively examine the data on the progress of this clinical trial, the eligibility evaluation results of the subjects, the safety, and efficacy. With the goal of ensuring the safety of the subjects, the Data Monitoring Committee will provide appropriate advice and recommendations to the investigator regarding the study continuation, changes, and interruption.

In particular, the scientific validity and ethical questions regarding the following matters will be comprehensively deliberated. Thereafter, both advice and recommendations will be given to the person in charge of implementing the study protocol:

- AEs that occur in this clinical trial and their measures
- The efficacy and safety of this clinical trial and measures and recommendations such as its continuation, changes, and cancellations as necessary
- Regarding the enrollment of the second case based on the evaluation of the first case after 12 weeks
- Regarding the enrollment of the third case based on the evaluation of the first and second cases after 12 weeks

The deliberation request made to the Data Monitoring Committee, the deliberation method, and the notification method for the deliberation results shall be conducted according to the standard operating procedure of the Data Monitoring Committee prepared for the trial.

2.10. Sample size and rationale for the setting

Since this clinical trial targets patients with familial LCAT deficiency, a rare disease, the target number of cases (number of administered cases) was set to three to ensure the feasibility of the clinical trial. The dose of the investigational products and study design were determined referring to the FIH clinical study under the Act on Securement of Safety cases) was set to three to ensure the feasibility of the clinical trial. The safety of the investigational products as well as effects of LCAT supplied from them on dyslipidemia caused by familial LCAT deficiency will be evaluated for the three cases in this study. Considering the clinical results of the case in the FIH clinical study, we considered that evaluating the safety, the exploratory efficacy, and the dosage to achieve a sufficient efficacy of the investigational products are all possible based on the target number of cases.

2.11. Population to be analyzed

1) Full analysis set (FAS)

The FAS is all subjects enrolled, treated with the investigational products, and with available efficacy data. However, subjects for whom baseline data have not been obtained and subjects who have seriously violated the protocol (such as the absence of informed consent) will be excluded.

2) Per protocol set (PPS)

Subjects conforming to the clinical trial protocol will be analyzed after subjects with the following serious violations of the provisions of the clinical trial protocol are excluded from the FAS: violation of inclusion criteria, exclusion criteria, concomitant prohibited drug administration, and concomitant prohibited therapy performance.

3) Safety analysis set

Cases enrolled in this study and for which adipose tissue was collected will be analyzed.

2.12. Data analysis plan

The analysis of the safety endpoints will be performed in the safety analysis set. The analysis of the efficacy endpoints will be performed in the FAS and PPS. This clinical trial will be conducted as a single-arm study, and in principle, a comparative analysis (statistical hypothesis test) and calculation of summary statistics will not be conducted due to the small number of cases.

1) Primary endpoint

Lists of the primary endpoints (RCR appearance, site tumorigenesis, anti-LCAT antibody appearance, and unexpected serious adverse events) will be created.

2) Secondary endpoints

Lists of adverse events and transition charts of clinical laboratory test values and vital signs for the secondary safety and efficacy endpoints will be created. Time-series transition charts of the observation period before and after administration will be created for each case, and a reference line will be added to compare the clinical values before and after administration, if necessary.

2.13. Interim analysis and monitoring

No interim analysis is planned.

2.14. Patient and public involvement

Neither the patients nor the public were involved in the design of this trial.

3. Discussion

This trial was planned to evaluate safety of the investigational product, LCAT gene-transduced autologous pre-adipocytes essentially. About 20 mutations have been identified for familial LCAT deficiency thus far in Japan [14], according to published literature; however, the number of patients who were introduced to Chiba University Hospital from other institutions as possible candidates is extremely limited. Thus, the target number of cases (number of administered cases) was set to three, with regulatory strategy consultation of the PMDA, based on the feasibility of enrollment in Japan. Although the degree of complications associated with the disease reported thus far has varied among the patients with familial LCAT deficiency, the degree of such complications in patients was not considered when establishing the inclusion criteria because of the limited number of patients included in this study. Therefore, the efficacy of the investigational product for all complications (including corneal opacity, proteinuria, and hemolytic anemia) may not have been sufficiently evaluated in this trial, as patients without some of the disease complications were enrolled.

The investigational product is an ex vivo retroviral vector-mediated...
gene therapy product, which must be prepared aseptically in a cell processing center compatible with the Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms. In addition, the products are required to be shipped and administered on the same day. Therefore, in case an urgent response to a patient is required, this study has been designed as a multicenter trial, with Chiba University Hospital administering and evaluating the safety/efficacy of the investigational product at all prescribed visits, and medical institutes other than Chiba University Hospital collecting information on AEs and concomitant medications/therapies between subject visits.

The endpoints for safety were set in relation to the characteristics of the investigational products. One unique point of this study is that the investigational products will be evaluated for tumorigenicity in immunodeficient mice in parallel with the clinical evaluation of the patients in this trial. Since the investigational product are intended to supply therapeutic wild-type LCAT protein, patients may generate antibodies against the LCAT protein as well as other ERTs. Patients who are not expected to express full-length LCAT protein due to frameshift or the appearance of a stop codon by nonsense or insertion/deletion mutations in the LCAT gene will be excluded from this clinical trial in order to minimize the risk of generation of antibodies against LCAT protein, since only two such mutations have been found among the approximately 20 mutations that have so far been previously identified in Japan [14].

The observation period was set to 24 weeks with reference to preclinical studies of the investigational products and prior FIH clinical study. It was considered possible to evaluate the safety based on the results of preclinical safety studies using healthy donor-derived human cells and animal cells that were similarly propagated. It was also considered possible to evaluate the exploratory efficacy endpoints by referring to time-series transition data of clinical tests related to those endpoints in prior FIH clinical study (manuscript submitted). Given the need to evaluate the long-term safety of gene/cell therapy products, the safety as well as exploratory efficacy will be confirmed in a subsequent clinical trial involving a long-term evaluation (total of 240 weeks).

While writing this manuscript, the first patient was registered at the end of December 2021. The investigational product will be administered to the first patient in January 2022. Finally, we hope that the treatment will be put into clinical practice in the near future to improve or even cure patients with LCAT deficiency. This novel adipocyte-based strategy is expected to facilitate the establishment of a new field of ex vivo gene therapy-mediated long ERT.

Ethics and dissemination

The trial will be conducted according to the principles of the World Medical Association’s Declaration of Helsinki and in accordance with Good Clinical Practice (GCP) standards. The protocol was approved by the institutional review board at Chiba University Hospital as well as the boards of five other institutions. Written informed consent will be obtained from all patients before they are enrolled. The trial findings will be published in a peer-reviewed journal.

Authorship

All authors had access to the protocol and Statistical Analysis Plan. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval to the version to be published.

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Author’s contributions

All authors have read and approved the manuscript. MK prepared the manuscript, which was edited by the authors (MH, YS, YO, YK, HH) from the Clinical Research Center. The protocol was developed with input from the PI (KY), sub-investigators (YM, YK, NM), and medical expert (YS).

Declaration of competing interest

MK has received joint research funding from CellGenTech, Inc., to conduct the FIH clinical study. HH has received joint research funding from CellGenTech, Inc., to conduct the clinical study described in the manuscript. KY has received joint research funding from CellGenTech, Inc., to develop the gene-transduced human pre-adipocyte.

Data availability

No data was used for the research described in the article.

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