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Remedial mechanism of simvastatin and ursodeoxycholic acid in liver cirrhosis: crosstalk of bile secretion, gut microbiome, and host Immune response

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Introduction

There is emerging evidence that the gut milieu plays an important role in the progression of complications of cirrhosis [1–3]. Studies have found dysbiosis in the gut microbiota in patients with cirrhosis that has the potential to influence complications such as hepatic encephalopathy [1,2]. The gut milieu in cirrhosis involves the interaction between the microbiota and secreted factors, such as bile acids (BAs) that can also modulate the gut barrier [4]. The gut microbiota is known to convert 7a-dehydroxylate primary BAs and chenodeoxycholic acid (CDCA) and cholic acid (CA) into the secondary bile acids lithocholic acid (LCA) and deoxycholic acid (DCA), respectively [5]. Cirrhotic patients have been shown to have a lower proportion of secondary Bas in their bile but the mechanism for this is not clear [6]. Since BAs have important downstream pathophysiologic effects, a better understanding of the interaction between the intestinal microbiome and BAs is important to gain insight into the pathophysiology of cirrhosis [7,8]. In Genta Kakiyama study[9], the median abundances of taxa are shown with significantly higher Enterobacteriaceae and Veillonellaceae and lower autochthonous bacteria (Blautia, Ruminococcaceae, Lachnospiraceae) in cirrhotics, especially advanced cirrhotics compared to healthy controls. The total bile acid was 206.5 μg/100 mg dry stool in healthy control, and 39.0 in advanced cirrhosis. The secondary BAs were present in a significantly lower percentage of cirrhotics. The ratios of all secondary to primary BAs, and specifically of DCA/CA and LCA/CDCA, were also the lowest in advanced cirrhosis. Cirrhosis dysbiosis ratio (CDR), the ratio of autochthonous/good bacteria (e.g. Lachnospiraceae, Ruminococcaceae and Clostridiales) to non-autochthonous/ pathogenic bacteria (e.g. Enterobacteriaceae and Streptococcaceae), is significantly higher in controls and patients with compensated cirrhosis than patients with decompensated cirrhosis. Incresing CDR could lead to develop the hepatic encephalopathy[10]. Bile acids are also the endogenous ligands of Farnesoid X receptor (FXR), Takeda G protein receptor 5 (TGR5) and Sphingosine-1-phosphate receptor 2 (S1PR2). These receptors are placed at the interface of the host immune system with the intestinal microbiota and are highly represented in cells of innate immunity such as intestinal and liver macrophages, dendritic cells and natural killer T cells[11]. In human study, ursodeoxycholic

acid (UDCA) could enhance the bile synthesis by CYP7A1 altering the gut microbiota population. UDCA also has other beneficial effects , such as anticholestatic, antifibrotic, antiproliferative, and anti-inflammatory [12-14].

In retrospective studies, statins appear to be safe in patients with compensated cirrhosis, and evidence suggests that they may significantly reduce fibrosis (HR:0.56, p<0.0001)[15]. Preclinical data from studies performed on in vitro and in vivo animal models shed light on favorable effects of statins on endothelial function, angiogenesis, PHTN, and fibrosis. Suggested mechanisms involve regulation of KLF2, eNOS, thrombomodulin, and C-natriuretic peptide. Animal studies evidence suggests that the administration of fluvastatin, lovastatin, and simvastatin to primary rat hepatocytes and human hepatoma cell line inhibits the paracrine activation of HSCs [16], reduces HSC turnover and activity [17,18]. Moreover, Nature (2020) reported statin therapy is associated with lower prevalence of gut microbiota dysbiosis. [19]

The combination of ursodeoxycholic acid and statins is safe in human experiments, and the combination of the two has also proved to have a synergistic effect on the reduction of bile cholesterol levels [20], but there is no research on improving fibrosis makers or improving intestinal flora.

The goal of the current study was to evaluate our hypothesis model of pathogenesis of chornic inflammation of liver cirrhosis. We hypothesized that an adverse microbiome would exacerbate the histopathological severity of liver induced by CCL4 in a germ-free mice model. We transplanted these mice with human fecal samples from healthy controls, or liver cirrhosis treated with staitn, or UDCA. The clinical trial mainly explores whether single statin or combined ursodeoxycholic acid can reduce liver fibrosis indicators, inflammatory cytokines and intestinal flora imbalance in patients with liver cirrhosis who have been cured of chronic hepatitis C or non-viral load hepatitis B.

Patients and assessments- Study design

We expect to enroll 120 stable patients who have achieved sustained viral response (SVR) (ie, viral eradication) after hepatitis C treatment for 6 months, or non-viral load hepatitis B for 6 months, who have been diagnosed with primary cirrhosis (FIB-4 ≥3.25) and met the Baveno VII criteria (liver stiffness measurement [LSM] ≥25 kPa) and exclude it (LSM <15 kPa and platelet count [PLT] ≥150×10^9/L) were used. Grey zones were classified into two groups: (high – LSM between 20–25 kPa and PLT <150×10^9/L, or LSM between 15–20 kPa and PLT <110×10^9/L, or low – defined as the remaining patients within the grey zone) [27]. **Exclusion conditions:** already taking statins, diabetes, liver cancer, alcoholic liver, moderate to severe fatty liver, liver decompensation (jaundice, ascites, esophagogastric varices, history of hepatic coma), chronic kidney disease, antibiotic use within three months and gastric ulcer medication(such as PPIs). Randomly divided into three groups: Group 1: control group (n =

30), Group 2: treatment group (ursodeoxy Cholic acid (UDCA) (10mg/kg/day) (n = 30), Group 3: treatment group (simvastatin 40mg) (n = 30), Group 4: treatment group (simvastatin 40mg/day + ursodeoxy Cholic acid (UDCA) (10mg/kg/day) (n = 30). Fecal bile acid content and fecal flora, skin bile acid profile, cytokine markers, biochemical fibrosis biomarker were collected at the test base point and six months after treatment. In addition, patients who achieved virus eradication without liver cirrhosis (FIB-4 < 1.45, n=30) collected the content of fecal bile acid, fecal flora, skin bile acid, and inflammatory cytokines were collected at the test base point index, biochemical fibrosis biomarkers. Analysis of flora: 16S ribosomal RNA gene sequence analysis from the patient's fecal tube. Analysis of total fecal bile acid: LC-MS/MS system analysis. Skin bile acid profile:Desorption-Electrospray Ionization Mass Spec-trometry (TD-ESI/MS).

Block randomization

Patients (n=120) were distributed on one block with 8 numbers and each group contained two numbers (1, 2, 3, 4). The admitted patients who were eligible for inclusion were given numbers in the order; number 1 was allocated to (control group) as the study group, patients who carried number 2 was allocated to (UDCA group),patients who carried number 3 was allocated to (simvastatin group), and number 4 was allocated to (simvastatin plus UDCA group). Therefore, the control and three interventional groups were randomized into 1:1:1:1.

Stool sample preparation and microbiota analysis

Bile juice and biliary stents samples will be collected in separate sterile containers containing 2mL of RNA later and will be stored at room temperature. The samples will be transferred to the laboratory within 12 h, stored at 4°C, and used for extracting DNA within few weeks. Preliminary experiments will confirm that the bacterial composition data does not change in four weeks under the storage conditions used. Genomic DNA will be isolated from stool samples using the CTAB/SDS methods (Novogene Laboratories) following the manufacture's protocol. All microbial community DNA samples will be analyzed for V4 variable region of bacterial 16S ribosomal RNA gene using PCR primers 515F and 806R. The PCR products will be separated by 2% agarose gel electrophoresis. Samples with bright main strip between 400-450bp will be chosen for further experiments. PCR product will be mixed in equidensity ratios. Then, mixture PCR products will be purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries will be generated using TruSeq DNA PCR-Free sample Preparation Kit (Illumina, USA) following manufacture's recommendations and index codes will be added. The library quality will be assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library will be sequenced on an IlluminaHiSeq2500 platform and 250 bp paired-end reads will be generated.

Fecal bile acid profile [20].

A stool sample was obtained and dried to obtain a lyophilized extract. To lyophilized faecal samples weighing 1 g, 80% methanol was added. All samples were sonicated for 30 min, refluxed for two hours, and then cooled and filtered. The residue was re-suspended in chloroform/methanol (1:1, v/v), refluxed for one hour, and filtered. The combined extracts were taken to dryness, and re-suspended in 10 ml methanol (MeOH). An aliquot of 1 ml was added with 2 µl of 1 mg/ml nordeoxycholic acid, and was diluted in 10 ml deionized water and deposed on a 300 mg HLB Oasis column, washed with 10 volumes deionized, 1 volume cyclohexane, and the BAs were then eluted with 5 ml MeOH and were taken to dryness and resuspended in 250 µl MeOH. Four microliters were injected on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) as previously described.

Skin bile acid profile [22]

TD-ESI source comprised of a direct probe (DP), thermal desorption unit, and electrospray ionization device. First, the direct probe (DP) was used for sampling analytes in solution or on solid surfaces. For liquid samples, a stainless steel inoculating loop serving as a probe (2 mm in diameter) was used to obtain approximately 2 µL of sample solution for each analysis. For viscous solutions or solid samples, a straight fine needle (350 µm in diameter) was used to either collect analytes of viscous solutions or sweep five times across solid surfaces to obtain analytes. Second, the function of the thermal desorption unit was to vaporize samples on the DP and introduce desorbed analytes into the ESI plume through a quartz tube with single taper (OD= 6 mm, ID= 4 mm, Length= 80 mm) so that effective sample ionization can take place. In addition, an opening in the thermal desorption unit was created when the direct probe was removed from the unit to sample analytes. The thermal desorption unit was sealed after the direct probe was placed back on the thermal desorption unit for analysis of sample analytes on the probe. Third, an electrospray ionization device was used for post-ionizing desorbed analytes. The high voltage and solvent flow rate required to generate an electrospray plume were 5 kV and 2.5 µL/min, respectively. The ESI solution was a methanol and water mixture (1:1, v/v) containing 0.1% acetic acid, and was delivered through a fused-silica spray capillary (ID= 100 µm, OD= 375 µm, Polymicro, Phoenix, AZ) into the mass inlet of the mass spectrometer. The TD-ESI source was attached to a triple quadruple mass spectrometer (Agilent 6410) to detect the analyte ions. To obtain a stable solvent and analyte ion signal, the electrospray capillary was aligned to the MS inlet and the distance from the exit of the TD tube to the electrospray capillary (*d1*) was kept at 8 mm, while the distance from the MS inlet to the electrospray capillary (*d2*) was 5 mm. The temperature in the thermal desorption unit was set at 250 °C and was measured by a thermistor installed in the source. The flow rate of the nitrogen gas used to deliver desorbed analytes into the ESI plume was 0.8 L/min.

Primary end point: Fibrosis biomarker [23]

Molecular markers of fibrogenesis including TGF-β1, and Type IV collagen. Hyaluronic Acid in the serum blood test, and clinical labotory FIB-4 Index.

Secondary end points: 1. Cirrhosis dysbiosis ratio (CDR), the ratio of autochthonous good bacteria(e.g. Lachnospiraceae, Ruminococcaceae and Clostridiales) to non-autochthonous/ pathogenic bacteria (e.g. Enterobacteriaceae and Streptococcaceae). 2. Total stool bile acid, Secondary and primary bile acid ratio. 3. Serum inflammation peptides (IL-6, IL-8, IL-10, and TNF-a)

Sample size : Till now, there was no prospective data to support the evaluation the sample size of study in the liver fibrosis deminition after the administrations of stain and UDCA. Prior data [24]may indicate the effect of 40 mg simvastatin versus placebo on hepatic portal pressure was studied over a period of 3 months. A significant decrease in HVPG (-15%) was noted in the experimental group versus no change in the placebo group. The study included 24 cases in each study arm. Therefore, we plan to enroll 30 participants in each arm.

Safety

Initial dose of study drug is 20 mg which can be increased to 40 mg after 4 weeks if the participant does not develop side effects. Creatine kinase and standard biochemistry including liver enzymes are measured for safety reasons after 2 weeks, to rule out myositis and liver damage. Side effects and events are consecutively registered throughout the trial. Safety of statins in chronic liver disease has been assessed in only a few clinical trials, but reviews of observational studies report high safety and low risk of adverse events, even significantly lower the all-cause mortality [25,26]

Statistical analysis:

Risks are reported as IRRs along with their 95% confidence intervals and *P* values. For subgroup analyses according to sample characteristics, *P* values for interaction also are assessed. The cumulative proportion of patients developing liver fibosis events during follow-up is estimated by life table methods and log-rank tests were reported. *P* values are 2-sided, and values of .05 or less are considered statistically significant. All statistical analyses are performed using SAS version 9.1 (SAS Institute Inc).

Limitation 1:

If post treated human feces contain the trace level of drug, the hypothesis that it is caused by microorganisms alone does not hold. We will check the post treated human feces by the TD-ESI (LTQ)for simvastatin (Formula: $C_{25}H_{38}O_5$, Exact mass: 418.57 Da),and Ursodeoxycholic acid (UDCA)(Formula : $C_{24}H_{40}O_4$. Exact mass : 392.29 Da). The pre-test was performed by TD-ESI (LTQ) as followed figures.

Limitation 2: The germ-free mice treated with carbon tetrachloride (CCL4) for 12 weeks for liver cirhosis model is novel test. We can not make sure the mortality rate under the

treament of CCL4. Germ-free mice are more vulnerable than normal mice. If high mortality of Germ-free mice was found, we will shift to normal mice treated with antibiotics before human fecal transplantation test.

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Chang Gung Memorial Hospital, Kaohsiung Branch

Chang Gung Medical Foundation

Informed Consent Form

I. Remedial mechanism of simvastatin and ursodeoxycholic acid in liver cirrhosis: crosstalk of bile secretion, gut microbiome, and host Immune response

II. Basic Study Information

Medical Record Number: _____

1. **IRB Approval Number: IRB 202201398A3**
2. **Study Site:** Chang Gung Memorial Hospital, Kaohsiung Branch
3. **Department Responsible for Execution:** Division of Gastroenterology and Hepatology
4. **Sponsoring Organization / Company:** Chang Gung Hospital Research Project
5. **Principal Investigator:** Dr. Chih-Ming Lian
   Affiliation: Division of Gastroenterology and Hepatology
   Title: Attending Physician (Lecturer Level)
   Phone Number: 0975-056-294

Co-Principal Investigator: Prof. We-Chun Leng
Associate Investigators: Prof. CHANG-CHUN HSIAO, Pro. Shiea Jentaie.
Affiliation: Division of Gastroenterology and Hepatology
Titles: Attending Physicians (Associate Professor Level, Assistant Professor Level, and General Attending Level)
Phone Number: +886-7-731-7123 ext. 2444
Emergency Contact Number for Participants: 0975-056-294

6. **Participant's Name:**
   Participant Study ID Number:
   Gender:
   Date of Birth:
   Mailing Address:
   Contact Phone Number:

III. Introduction

Hello. Currently, global treatment outcomes for hepatitis B and C have been quite successful. However, for patients with liver cirrhosis who have already achieved viral eradication, underlying inflammation often continues to progress. At present, clinical management is limited to observation and monitoring.

Recent studies have found that bile secretion in cirrhotic patients is reduced to between one-fifth and two-thirds of that in healthy individuals. This bile acid deficiency leads to gut microbiota imbalance, which in turn increases inflammatory markers and may worsen cirrhosis or even contribute to the development of liver cancer. Therefore, the development of preventive therapies is extremely important.

In previous animal studies of liver cirrhosis, bile acid supplementation alone was shown to improve dysbiosis, reduce gut inflammation, and regulate intestinal immunity. Ursodeoxycholic acid (UDCA), an approved treatment for cirrhosis, promotes the secretion of endogenous bile acids and compensates for bile acid deficiencies in cirrhosis. Both human and animal studies have demonstrated its anti-inflammatory, anti-fibrotic, and

metabolic-regulating effects.
Statins, based on retrospective studies, have been associated with a reduced risk of liver fibrosis in patients. Animal research has also shown that statins can directly inhibit hepatic stellate cell activation and the differentiation of myofibroblasts, which are key processes in liver fibrosis. However, no prospective human trials have yet validated these findings.
The combination of UDCA and statins has been proven safe in human studies, and the two drugs together show a synergistic effect in reducing bile cholesterol content. However, no prospective studies have investigated their combined effect on improving liver fibrosis or gut microbiota in cirrhotic patients.
Therefore, this study aims to evaluate whether simvastatin alone or in combination with UDCA can reduce liver fibrosis markers, inflammatory cytokines, and gut microbiota dysbiosis in patients with liver cirrhosis who have been cured of chronic hepatitis C or have non-replicating hepatitis B.
Before agreeing to participate in this study, your physician will explain the contents of this informed consent form to you. Please read it carefully and feel free to ask any questions. Participation in this study is completely voluntary. Declining to participate will not affect your right to receive proper medical care.

IV. Purpose of the Study

To evaluate whether simvastatin alone or in combination with ursodeoxycholic acid (UDCA) can reduce liver fibrosis markers, pro-inflammatory cytokines, and gut microbiota dysbiosis in patients with liver cirrhosis.

V. Inclusion and Exclusion Criteria

Inclusion Criteria:

You are eligible to participate in this study if all of the following conditions are met:

1. Adults aged 18 to 75 years
2. Diagnosed with primary liver cirrhosis (FIB-4 ≥ 3.25)
3. Liver stiffness measurement (LSM) ≥ 25 kPa, or classified into the Baveno VII "grey zone"
4. Achieved sustained virological response (SVR) at least 6 months after hepatitis C treatment, or
5. Non-replicating hepatitis B infection (undetectable viral load) for at least 6 months
6. Able and willing to provide informed consent

Exclusion Criteria:

You will not be eligible to participate in this study if any of the following conditions apply:

1. Current or prior use of statins
2. Liver decompensation (jaundice, ascites, hepatic coma, or esophagogastric varices)
3. Diagnosed hepatocellular carcinoma or other liver cancers
4. Alcoholic liver disease or moderate-to-severe fatty liver
5. Diagnosed diabetes mellitus
6. Chronic kidney disease
7. Use of antibiotics within the past 3 months
8. Use of gastric ulcer medications such as proton pump inhibitors (PPIs)
9. Pregnancy or breastfeeding
10. Any condition deemed by the investigator to interfere with study participation or outcomes

VI. Study Methods and Procedures

Between **November 2022 and December 2025**, we plan to recruit **120 stable patients with liver cirrhosis** at **Kaohsiung Chang Gung Memorial Hospital, Taiwan**. Eligible participants include those who have achieved a **sustained virologic response (SVR)** for at least 6 months after completing treatment for **hepatitis C**, or those with **hepatitis B** who have had **undetectable viral load for at least 6 months** while on antiviral therapy, and have been diagnosed with cirrhosis (**FIB-4 ≥ 3.25**).

Participants will be randomly assigned to one of four groups:

1. **Group 1: Control group** (n = 30) – no active treatment
2. **Group 2: UDCA group** (n = 30) – receives ursodeoxycholic acid (UDCA) at 10 mg/kg/day
3. **Group 3: Simvastatin group** (n = 30) – receives simvastatin at 40 mg/day
4. **Group 4: Combination group** (n = 30) – receives both simvastatin (40 mg/day) and UDCA (10 mg/kg/day)

At baseline and after 6 months of treatment, we will collect:

- Fecal samples for **bile acid concentration** and **microbiota analysis**
- Skin swabs for **bile acid profiling**
- Blood samples (10–15 mL) for **inflammatory cytokines** and **biochemical fibrosis markers**
- Non-invasive **liver stiffness measurements** using transient elastography

In addition, we will collect the same set of baseline and 6-month samples from **30 non-cirrhotic patients** who achieved **SVR after hepatitis C** and have a **FIB-4 score < 1.45**. These participants will undergo:

- Two fecal samples for **bile acid and microbiota analysis**
- Skin swabs (collected by gently rubbing a small steel ring on the forehead 2–3 times, non-invasive and does not cause injury)
- Blood draw (10–15 mL) for **cytokine and fibrosis marker analysis**
- **Liver stiffness measurement** using a non-invasive, ultrasound-based device

VII. Potential Risks, Side Effects, Incidence Rates, and Management

In general, **simvastatin is well tolerated**, and most of its side effects are **mild and temporary** in nature. Controlled clinical trials have shown that **fewer than 2%** of patients discontinue simvastatin due to adverse effects.

Reported side effects include:

- **Abdominal pain, constipation, and bloating** (approximately 1%)
- **Fatigue and headache** (0.5–0.9%)
- **Myopathy** (muscle-related side effects) are **rare**, occurring in **less than 0.1%** of patients
- **Elevated liver transaminases** confirmed to be more than **3 times the upper limit of normal** on repeated testing in **0.21%** of patients

For **ursodeoxycholic acid (UDCA)**, the **overall incidence of side effects** is around **2.6%**, most of which are gastrointestinal in nature:

- **Soft stools, diarrhea, nausea, and vomiting** occur in approximately **2.26%**
- **Allergic reactions**, such as **itching or rash**, occur in **0.22%**
- **Other rare adverse effects** account for **0.1%**

Management Procedures:

1. If you experience any discomfort, please inform your attending physician. They will provide you with the most appropriate care and treatment.
2. In the event of an emergency or any unusual symptoms that cannot be controlled with standard medical treatment, please contact your physician **Dr. Chih-Ming Liang** at **0975-056-294** or the research staff at **07-342-2121 ext. 2444** immediately.

VIII. Alternative Treatment Options

For patients with liver cirrhosis who have achieved viral eradication of chronic hepatitis B or C, current clinical guidelines recommend **biochemical testing and liver ultrasound every 3 to 6 months for monitoring**, but **do not include any specific medication recommendations** for ongoing treatment.

IX. Contraindications and Restrictions Related to This Study

This study will be conducted in conjunction with your scheduled follow-up visits and standard medical care. **You will not be required to follow any additional restrictions.**

X. Expected Outcomes of the Study

In patients with liver cirrhosis, **bile acid supplementation or combination therapy with simvastatin** may help improve the **gut microbiota composition**, potentially **restore total bile acid levels**, and lead to a **reduction in pro-inflammatory cytokines** and **biochemical fibrosis markers**. However, further studies are needed to confirm these effects.

XI. Emergency Management

If you experience any discomfort or adverse symptoms during the study, please inform your **study physician** immediately. Your doctor will make every effort to provide the most appropriate care and treatment.

In the event of a **medical emergency** or **unusual physical condition** that cannot be effectively managed with standard medication, please contact your **attending physician** or the **Principal Investigator**, **Dr. Chih-Ming Liang (Division of Gastroenterology and Hepatology)**, at his **24-hour emergency contact number: 0975-056-294**, or contact the study staff at **+886-7-731-7123 ext. 2444**.

XII. Compensation, Cost Responsibility, and Injury Reimbursement

1. **Compensation:**
   There is **no monetary compensation** provided for participation in this study.
2. **Cost Responsibility:**
   This study is funded by research grants from Chang Gung Memorial Hospital. Under Taiwan's National Health Insurance system, **you will not incur any additional costs** for participating in this study.

(1) If an adverse event or injury occurs as a direct result of procedures conducted according to the study protocol, the hospital and the principal investigator will bear legal responsibility for providing compensation, **except for adverse events that are already listed as foreseeable in this consent form**, which will not be eligible for compensation.

(2) In the event of an adverse reaction or injury related to this study, **professional medical care and consultation will be provided at no cost to you**.
(3) Aside from the above-mentioned compensation and medical care, **no additional compensation will be offered**. If you are not comfortable with this level of risk or arrangement, you are advised **not to participate in this study**.
(4) Signing this informed consent form **does not waive any of your legal rights**

XIII. Privacy and Confidentiality Protection

1. A unique **research code** will be assigned to represent your identity. This code will **not contain your name, national ID number, or address**.
2. All diagnostic information and study findings will be handled with strict confidentiality by the principal investigator. If research results are published, **your personal identity will not be disclosed**.
3. By signing this consent form, you agree that your research records may be directly accessed by authorized monitors, auditors, the Research Ethics Committee (IRB), and regulatory authorities **to ensure compliance with applicable laws and regulations**. These individuals are obligated to **maintain the confidentiality of your personal information**.

XIV. Withdrawal and Discontinuation from the Study

You, or your legal representative, **have the right to withdraw from this study at any time and for any reason** without affecting your medical care or legal rights.
Additionally, the **principal investigator may terminate your participation** or halt the study if necessary.

XV. Subject Rights

1. The collection, processing, and use of your personal data will be handled by the study institution and investigators in accordance with this informed consent form, relevant clinical trial regulations, and the **Personal Data Protection Act** of Taiwan. You may exercise the following rights in writing by contacting the study institution or investigator:

(1) Request to access or review your personal data;
(2) Request a copy of your personal data;
(3) Request to supplement or correct your personal data;
(4) Request to stop the collection, processing, or use of your personal data;
(5) Request deletion of your personal data.

2. During the course of the study, you will be promptly informed of any significant new findings that may affect your willingness to continue participation. If you have any questions or concerns during the study, please contact the principal investigator.
3. If you have questions about your rights as a research participant or believe that you have been harmed by participating in this study, you may contact the **Institutional Review Board (IRB)** of this hospital for consultation at:
   (03) 319-6200 ext. 3703, 3705–3709, 3711–3717

XVI. Ownership of Study Results and Benefits

If the results of this study lead to academic publications, tangible benefits, or other related outcomes, you agree that such outcomes may be donated **free of charge** to this hospital for **public interest purposes**, such as disease prevention, diagnosis, and treatment.

XVII. Storage and Reuse of Personal Data, Samples, and Derivatives

This study **does not involve any leftover biological samples** for future storage or reuse.

XVIII. Declaration

The contents of this study and the informed consent form have been **fully explained orally** to the participant by _____. The participant and/or their legal representative has fully understood and agrees to participate in the study.
This consent form is made in **duplicate**, and a **copy has been provided to the participant**.

Signatures

A. Participant:

Name (Print): _____

Signature: _____

Date: ____ / ____ / _____ (YYYY/MM/DD)

B. Person Obtaining Consent:

Name (Print): _____

Signature: _____

Date: ____ / ____ / _____ (YYYY/MM/DD)

C. Co-Investigator / Associate Investigator:

Name (Print): _____

Signature: _____

Date: ____ / ____ / _____ (YYYY/MM/DD)

D. Principal Investigator:

Name (Print): _____

Signature: _____

Date: ____ / ____ / _____ (YYYY/MM/DD)

(*Required if the participant meets the condition described in Section I of the Consent Signing Instructions*)

E. Legal Representative / Authorized Consenter / Guardian / Conservator:

Name (Print): _____

Signature: _____

Date: ____ / ____ / _____ (YYYY/MM/DD)

Relationship to the Participant: _____

(*Required if the participant meets the condition described in Section II of the Consent Signing Instructions*)

F. Witness:

Name (Print): _____

Signature: _____

Date: ____ / ____ / _____ (YYYY/MM/DD)

[Chang Gung Medical Foundation / Chang Gung University / Chang Gung University of Science and Technology – Information for Research Participants]

Dear Participant, Family Member, or Member of the Public,
If you meet the eligibility criteria for a clinical trial or research study, you may be invited to participate in a study conducted by Chang Gung Memorial Hospital, Chang Gung University, or Chang Gung University of Science and Technology. The following information is provided to protect your safety and rights as a research participant. It explains the role and responsibilities of the **Institutional Review Board (IRB)** at Chang Gung Medical Foundation, how research is reviewed, and what your rights are as a participant.

What is Research?
Research is different from treatment.
Treatment involves procedures and medications that have already been studied and are known to have predictable outcomes and side effects.
Research, on the other hand, aims to discover new knowledge and often involves **uncertainty about outcomes**. Participation in research is **completely voluntary**, and choosing not to participate **will not affect your right to receive medical care** or result in any form of unfair treatment.

What is the Institutional Review Board (IRB)?
The **Institutional Review Board (IRB)** is a committee established to ensure that research involving human participants is **scientifically and ethically appropriate**.
It is composed of medical professionals, legal experts, community representatives, and members of non-medical backgrounds who help researchers understand the concerns of participants to ensure their safety and rights are protected.
If you have any questions about your rights, you may contact the IRB of Chang Gung Medical Foundation.

How Does the IRB Review Clinical Trials or Research Studies?
1. **All research conducted at Chang Gung Memorial Hospital, Chang Gung University, or Chang Gung University of Science and Technology must be reviewed and approved by the IRB** before it can begin.
2. Research protocols submitted to the IRB are independently and professionally reviewed by committee members and experts.
   The review focuses on whether participants are fully informed about the study — including:
   - The purpose of the study
   - The procedures involved
   - Possible alternative treatments

- Potential risks, side effects, and benefits
- How to withdraw from the study
- How privacy and confidentiality will be protected

3. During review, the IRB evaluates **potential risks** to participants, which may include:
   - Physical discomfort or harm
   - Psychological stress
   - Social or financial impact

The IRB ensures that all **risks are minimized** and justified by the **potential benefits** — either to the participant, or to future patients through scientific knowledge.
Studies that pose high risk with **no reasonable benefit** will **not be approved**.

4. After a study is approved, the IRB and the research institution will **continue monitoring the research** to ensure that it is carried out exactly as approved and that participants' rights and safety remain protected.

What Are Your Rights as a Research Participant?

- **Right to Be Informed**

You have the right to clear and complete information about:

1. **Purpose of the study**
   The researcher must explain the aim of the study in simple and understandable language.
2. **What will happen during the study**
   You should know what procedures will be involved, how often visits occur, whether blood samples will be taken, and any inconvenience to your daily life.
3. **Alternative treatment options**
   You have the right to know about other treatments available if you choose not to participate.
4. **Risks and side effects**
   All research involves risk. You must be informed of potential risks and side effects, and what to do in case of an emergency — including who to contact and who will provide follow-up medical care and cover related costs.
5. **Potential benefits and expected outcomes**
   Researchers must explain any direct benefits to you, or how the study may help others in the future through new discoveries.
6. **How to withdraw from the study**
   You must be informed about how to withdraw at any time, what happens to your data after withdrawal, and whether follow-up care is available.
7. **Freedom to ask questions**
   You may ask the research team questions **at any time**.

- **Right to Voluntary Participation**

You will only officially become a participant **after the researcher explains** the study purpose, procedures, risks, benefits, and your rights, and **after you sign the informed consent form** of your own free will.
You may also **withdraw at any time** without giving a reason.
Your decision to withdraw will **not affect your future medical care** or cause you to be treated unfairly.

- **Right to Protection**

1. **Privacy and confidentiality**
   Any personal information collected during the study will be kept confidential.

If study results are published, or reviewed by the IRB or regulatory agencies (e.g., the Ministry of Health and Welfare), **your identity will not be revealed**.

2. **Retention of your legal rights**
   Participation in a clinical study does **not mean you give up any legal rights** you currently have

Chang Gung Medical Foundation

Institutional Review Board

199, TUNG HWA NORTH ROAD,

TAIPEI, TAIWAN, 10507

REPUBLIC OF CHINA

Tel: (03) 3196200

Fax: (03) 3494549

Date 2022/11/01

Protocol Title: Does the Combination of UDCA and Statin Improve the Dysbiosis and Diminish the Fibrosis biomarker of the HCV patients with advanced fibrosis after Sustained Virological Response?

IRB No.: 202201398A3

Principal Investigator(s): LIANG, CHIH-MING

Co-Investigator(s): CHUAH SENG KEE, HSIAO, CHANG-CHUN, TSAI, MING-CHAO, CHANG, KUO-CHIN

Executing Institution: Kaohsiung

Duration of Approval: From 2022/11/1 To 2023/10/18

Version/Date of documents:

(1)Protocol: 2022/08/15 Version1

(2)Chinese Synopsis: 2022/08/15 Version1

(3)Informed Consent Form: 2022/10/31Version4

(4)Case Report Form: 2022/08/15 Version1

Date of Meeting: 2022/10/19

Date of Approval: 2022/11/01

Frequency of Continuing Report: Once a year

※Next Deadline of Continuing Report: 2023/10/18. To facilitate the review, please submit the report two months before the deadline (or one month before the expiration of the trial if a continuing report shall be provided every three months) in order not to influence the principal investigator's right to conduct the research. In the case that failure or delay to submit a continuing report makes the IRB unable to determine the next trial period by the deadline, the trial shall not be continuously conducted. If the Principal Investigator fails to submit a continuing report on time, rendering the Institutional Review Board unable to issue the next trial execution period before the previous trial execution period expires, all research activities, including the intervention or interaction with

the participating trial subjects, must be suspended. Unless the Institutional Review Board considers that the continuation of trial intervention or trial participation is greatly beneficial to the trial subject's safety or in the best interest of the trial subject from a moral point of view, no new trial subject shall be included until the continuing report is formally approved.

The IRB is organized and operates in accordance with Good Clinical Practice and the applicable laws and regulations.

T. T. Hsieh

Tsang-Tang Hsieh,MD
Chairman
Institutional Review Board
Chang Gung Medical Foundation