MICROB-PREDICT

MICROBiome-based biomarkers to PREDICT decompensation of liver cirrhosis and treatment response

D7.1 Final version of study protocol to be approved by ethics committee(s)

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Abbreviations

EFCLIF	European Foundation For The Study of Chronic Liver Failure
CEA	Commissariat à l'Energie Atomique et aux Energies Alternatives
ICN2	Fundacio Institut Catala De Nanociencia i Nanotecnologia
UNIDEB	Debreceni Egyetem (University of Debrecen)
OUH	Odense Universitetshospital
GUF	Johann Wolfgang Goethe-Universität Frankfurt Am Main
KUL	Katholieke Universiteit Leuven
LUMC	Academisch Ziekenhuis Leiden
UB	Universitat de Barcelona
UCL	University College London
ELPA	European Liver Patients Association
KCL	King's College London
FCRB	Fundacio Clinic Per A La Recerca Biomedica
EASL	European Association For The Study of the Liver
SEAB	Scientific and Ethical Advisory Board
GA	General Assembly
DSMC	Data and Safety Management Committee
ITF	Innovative Task Force
EMA	European Medicines Agency
MELD	Model for End Stage Liver Disease
TIPS	Transjugular Intrahepatic Portosystemic Shunt
LTx	Liver transplantation
AKI	Acute Kidney Injury
HRS	Hepatorenal syndrome
HE	Hepatic encephalopathy
CTMC	Clinical Trial Management Committee

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1. Executive Summary

The deliverable of having a study protocol ready to approval by ethical committees is done in time. The work around the study protocol has included several steps departing from the clinical trial template provided with the MICROB-PREDICT Grant Agreement. OUH provided the first version of the study protocol to review for the other study sites involved in Alb-Trial (GUF, KCL, UCL, KUL, UNIDEB, FRCB and LUMC) and other parties involved in the WP (EF-CLIF and ELPA). After refinements and corrections were performed, key opinions leaders have been consulted through the MICROB-PREDICT SEAB for their feedback and a meeting with the European Medical Agency was held to get a regulatory perspective on the study protocol and study design.

2. Deliverable report

The 7.1 deliverable of MICROB-PREDICT is achieved on time. The deliverable is a product of Task 1 and 2 in WP7 which are described as:

Task 1: Finalise study design

"… OUH will be responsible for the study design with support from EFCLIF and GUF regarding which tools to be validated, interpretation of results from the applied biomarkers and biomonitors and selection of appropriate cut-offs for selecting patients likely to benefit from treatment" (MICROB-PREDICT DoA)

Task 2: Obtain ethical approvals and prepare study launch

"The goal of this task is to ensure the foundation for a well-organised clinical trial. OUH, EFCLIF, GUF will finalise the study protocol with input from participating partners, ELPA and UB after which each center will submit applications to obtain all the necessary approvals. This includes e.g. ethical approvals, data protection regulations, good clinical practice and approval from national medicines agencies. We will also ensure an optimal, uniform trial infrastructure including setup of an eCRF hosted by EFCLIF with internal and external data validation and a data management plan. EFCLIF will also organise the human albumin infusions to be used as study intervention. Before the start of the study, training will be conducted for all clinical staff in order to make sure that measures are taken in a similar way and data quality will be comparable." (MICROB-PREDICT DoA)

2.1 Work with the study protocol

The clinical trial template provided as part of the MICROB-PREDICT Grant Agreement served as the steppingstone for the final protocol (MICROB-PREDICT Grant Agreement). Since the inception of MICROB-PREDICT the study protocol has been through 10 revisions to ensure that the final protocol adheres to the current best practice with use of novel methodological approaches to test predictive biomarkers. The work with the study protocol has been a

continuous and iterative process where input and suggestions from **internal- and external parties** have provided the basis for the final version.

2.2 Consultations with internal parties in MICROB-PREDICT

Study sites

Participating study sites in Alb-Trial (clinical study) are UNIDEB, GUF, KUL, KCL, UCL, FCRB, LUMC and OUH. During the study protocol work process all sites have been included in the discussion about recruitment, in- and exclusion criteria and endpoints. The annual general assemblies (GA) in MICROB-PREDICT (2019 Barcelona, 2020 Frankfurt and 2021 virtually) have served as the forum for this discussion. In addition, a virtual meeting for all WP7 partners was held in June 2020 to facilitate a final discussion where all study sites agreed on the main aspects of the trial.

CTMC

The work with study protocol has been governed by the Clinical Trial Management Committee. Members of the CTMC are Jonel Trebicka (KGU), Pere Ginés (FCRB), Maria Papp (UNIDEB), Minneke Coenraad (LUMC), Aleksander Krag (OUH) and Paolo Angeli (University of Padova). Input for the study protocol was provided from the CTMC at The Liver Meeting in Boston November 2019 where especially in- and exclusion criteria together with secondary endpoints were discussed.

EF-CLIF

During the process of refining the protocol, input from data managers and statisticians at EF-CLIF have concerned the data collection and quality assurance together with stopping rules for the Data and Safety Management Committee (DSMC).

2.3 Consultations with external parties outside MICROB-PREDICT

EMA

Apart from the ethical approval, regulatory approval is also needed due to the use of human albumin and the MICROB-PREDICT biomarker. The initial contact was made from OUH to the National Competent Authority in Denmark (“Lægemiddelstyrelsen”) to seek advice on the trial design from a regulatory point of view. However, due to the multinational setup, novelty of the biomarker and study design, questions were referred to European Medical Agency (EMA). A request for a Brief Meeting with the Innovative Task Force (ITF) within EMA was made in October 2020. The request was approved, and the meeting was held in

February 2021 with input from WP5 partners (CEA and ICN2) in MICROB-PREDICT. The meeting report can be seen in Appendix 1, dated 03.04.2021.

SEAB

In May 2020, a virtual SEAB meeting was held with the following participants from the SEAB: Bernd Schnabl, Vijay Shah and Tilman Sauerbruch. During the meeting, OUH presented the envisioned trial for the SEAB where emphasis from the SEAB was put on the outcome assessment, biomarker, primary endpoint and recruitment mitigation measures.

ELPA event

To include a non-scientific audience in the process of the development of the protocol, the study design was presented at the European Liver Patients' Association (ELPA) event in July 2020. The event was open for members of the national liver patients' association and provided an informal platform for any questions that potential participants may have. Furthermore, as a contributing partner in MICROB-PREDICT, ELPA has provided input to the study protocol during the General Assembly meeting in Frankfurt in January 2020.

2.4 Major revisions to the final protocol

Based on all the consultations and discussions mentioned above, a range of revisions have been made to the version of the protocol ready for ethical approval. Three areas of the protocol have undergone major alterations during this process. These are (1) primary endpoint, (2) in- and exclusion criteria and (3) secondary endpoints.

2.4.1 Primary endpoint

The primary endpoint of the study was changed since a confounding issue of using a >3 point increase in MELD score was identified. Since human albumin binds non-conjugated bilirubin, which is part of the MELD score, the use of a change in MELD score was deemed unsuitable for the study. Instead, the **cumulative number of liver-related clinical outcomes** was preferred after consultations with leading experts in the field. The primary endpoint model depicted in Figure 1 is a **recurrent event count model** of the following events:

- Decompensation episodes
- Death
- Transjugular intrahepatic portosystemic shunt (TIPS)
- Liver transplantation (LTx)

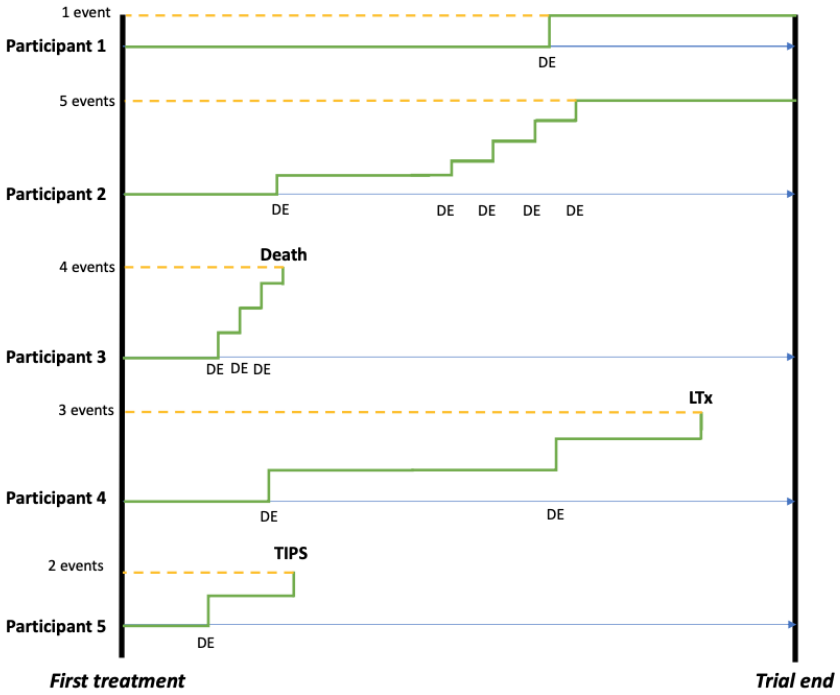


Figure 1 - Primary endpoint model

In the model TIPS and LTx serve as counting and censoring events. Decompensation episodes are defined as below. Detailed definitions of the events can be found in the attached study protocol (Appendix 2, version 1.0).

- Variceal bleeding
- Ascites
- Spontaneous bacterial peritonitis
- Infection requiring hospitalization
- Acute kidney injury (AKI) or HRS
- Overt hepatic encephalopathy (HE)

2.4.2 Inclusion and exclusion criteria

The in- and exclusion criteria of the study population have been redefined to ensure a representative population of patients without too severe competing co-morbidities.

Inclusion criteria

- Decompensated liver cirrhosis defined as Child Pugh class B or C and/or MELD ≥ 15

- Clinical and/or ultrasound evidenced ascites

- Age ≥ 18 years

- At least five days since resolution of a decompensation event or any condition requiring hospitalization

Exclusion criteria

- Patients with acute or subacute liver failure without underlying cirrhosis
- Patients with cirrhosis who develop decompensation in the postoperative period following partial hepatectomy
- Refractory ascites
- Existing TIPS
- Portal vein thrombosis
- Full stratification group
- Severe alcoholic hepatitis (Glasgow Alcoholic Hepatitis Score > 11)
- ≥ Child Pugh C13
- Current, planned or previous treatment with direct antiviral agents for HCV in the last six months
- Contraindications for human albumin infusion (pulmonary oedema, hypersensitivity etc.)
- Evidence of current malignancy expect for non-melanocytic skin cancer and hepatocellular carcinoma within BCLC-0 or BCLC-A
- Presence or history of severe extra-hepatic diseases (e.g., chronic renal failure requiring hemodialysis, severe heart disease (NYHA > II); severe chronic pulmonary disease (GOLD Score ≥ C), severe neurological and psychiatric disorders, pulmonary arterial hypertension)
- HIV positive or other condition associated with and/or requiring immunosuppression
- Previous liver or other transplantation
- Pregnancy
- Breastfeeding
- Patients who decline to participate or who cannot provide prior written informed consent and when there is documented evidence that the patient has no legal surrogate decision maker and it appears unlikely that the patient will regain consciousness or sufficient ability to provide delayed informed consent
- Physician's denial (investigator considers that the patient will not adhere to the study protocol scheduled, e.g. in case of heavy drinking)

The refined in- and exclusion criteria have been decided upon after meetings with WP7 partners (other study sites) and performed in alignment with position paper on endpoints and design of clinical trials in patients with decompensated cirrhosis[1].

2.4.3 Secondary endpoints

After consultation with the CTMC and SEAB several secondary endpoints have been added to the study protocol to analyze other aspects of interest. The secondary endpoints in the final protocol are the following:

- 6-months survival
- The number of episodes of acute-on-chronic liver failures
- Number of organ failures
- Time-to-liver-related clinical outcome
- Patients' Quality of Life
- Time to first hospital admission (in days)
- Number of hospital admissions
- Days spent on hospitalization (in days)
- Number of intensive care unit admissions
- Length of intensive care unit admission (in days)
- Number of large volume paracentesis
- Analysis of the cost/effectiveness ratio
- Health economic evaluation
- Changes in serum albumin levels
- Number of treatment-related adverse events
- Number of treatment-related serious adverse events
- Signatures associated with a poor prognosis as defined by the MICROB-PREDICT biomarker

2.5 Approval process

It is the goal to obtain the final ethical approval in Autumn 2021. The ethical approval is expected to happen in a sequential fashion with approval at some sites first (OUH, KGU) while others will follow shortly hereafter. The regulatory and data protection approval will follow soon after the ethical approval. Due to an adaptable study design and a well-characterized patient population, any of the biomarkers that are currently undergoing investigations in the MICROB-PREDICT consortium will be suitable for the Alb-Trial study with the aim of biomarker validation.

[1] Solà E, Pose E, Campion D, et al. Endpoints and design of clinical trials in patients with decompensated cirrhosis: Position paper of the LiverHope Consortium. J Hepatol. 2021;74(1):200-219. doi:10.1016/j.jhep.2020.08.009

3. Acknowledgement and Disclaimer

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This report reflects only the author's view and the Commission is not responsible for any use that may be made of the information it contains.

03.04.2021

ITF Briefing meeting report

Centre for Liver Research - Odense University Hospital

Briefing meeting held with the European Medicines Agency (EMA) on 24.02.2021, 14:05 – 15:39h.

The objective of the ITF briefing meetings is to provide for a preparatory discussion on scientific and regulatory topics relevant to the development of new medicinal products and technologies complementing and reinforcing existing formal procedures.

Proposed trial name:	**MICROB-PREDICT ALB-Trial**
Applicant:	MICROB-PREDICT Consortium, represented by Odense University Hospital
Proposed active substance:	Albumin (already approved for this indication and recommended by EASL guidelines)
Proposed finished product:	Nanobiosensors connected to smartphone measuring new biomarkers on a per-patient basis to stratify a) who is at risk for progression of disease ('personalized healthcare') and b) who will benefit from Albumin
Proposed indication:	Decompensated cirrhosis and acute-on-chronic liver failure (ACLF)

Participants

Applicant participants:

Name	**Organisational entity**
Jonel Trebicka	Goethe University Frankfurt, European Foundation for the Study of Chronic Liver Failure
Esaú Moreno	European Foundation for the Study of Chronic Liver Failure
Aleksander Krag	Odense University Hospital
Mark Ainsworth	Odense University Hospital
Nikolaj Christian Torp	Odense University Hospital
Mads Israelsen	Odense University Hospital
Christophe Junot	Commissariat à l´énergie atomique et aux énergies alternatives
Arben Merkoci	Institut Català de Nanociència i Nanotecnologia
Ameli Schwalber	concentris research management GmbH

List of EMA participants:

Name	Organisational entity
Klaus Kruttwig	Research and Innovation
Anthony Humphreys	Head of Regulatory Science and Innovation Task Force
Jordi Llinares Garcia	Head of Research and Innovation
Natalie Hendrikse	Research and Innovation
Armin Ritzhaupt	Digital Business Transformation Task Force
Thorsten Vetter	Scientific Advice
Katarina Vucic	Therapies for endocrine and cardiovascular diseases
Marcia Rueckbeil	Methodology

List of Experts:

Name	Committee or Working Party	NCA
Viktoriia Starokozhko		CBG/MEB
Johannes Taminiau	Gastroenterology Drafting Group	CBG/MEB
Elmer Schabel	Gastroenterology Drafting Group	BfArM
Andreas Kirisits	SAWP	AGES
Stephan Lehr	BSWP	AGES
Ferran Torres	BSWP	AGEMED/AEMPS

Disclaimer presented at beginning of meeting

The views expressed in this document are the opinion of the participating members of the Innovation Task Force and the experts and may not reflect the opinion of the EMA scientific committees. Therefore, the answers provided should not be interpreted as regulatory guidance or review recommendations for an application, but as a preliminary set of scientific considerations of the information presented.

Should aspects of the subject matter discussed herein become part of a formal data submission, application, or supplement, it is at the full discretion of the appropriate working party, evaluation team or scientific committee to completely and independently assess the product(s)/technology(ies) in question.

1. Background

Decompensation of liver cirrhosis and ACLF development constitutes a global health problem. The European Commission funded MICROB-PREDICT consortium investigates the human microbiome to identify predictors and mechanisms associated with the development of decompensation and progression to ACLF and death.

Our ambition is to find predictive biomarkers and evaluate these following the classical/conventional methods. Discovering of the biomarkers will be followed by development of the corresponding receptors (development of antibodies if not commercially available, and their proper evaluation). It is our goal to **design, validate and implement three microbiome-based tests** (to detect risk of progression to ACLF, response to treatment of Albumin, response to treatment of Rifaximin) as personalized medicine approaches to maximize the effectiveness of health care and therapies, thereby decreasing the rate of progression towards decompensation and ACLF, providing focused care of high-risk patients and reducing mortality. The tests shall be designed as **point-of-care tests for use by clinicians and patients**, based on **nanotechnology connected to a smartphone** application for data interpretation.

We are proposing to **validate the predictive biomarkers and biosensors in a randomised clinical trial** ("Alb-Trial"), in patients with decompensated cirrhosis stratified by the expected treatment response to albumin. This will ensure a personalized approach to long-term human albumin administration where patients believed to benefit will receive treatment and those not expected to benefit will be saved from the potential side-effects. (Note: Response to treatment of Rifaximin is not subject of the proposed trial.)

2. Summary of applicant presentation

Jonel Trebicka started off by introducing the people on the call and explaining the vision of MICROB-PREDICT. He elucidated the disease, decompensation of cirrhosis, eventually progressing to acute-on-chronic liver failure, its clinical features and implications. MICROB-PREDICT searches for microbiome-based biomarkers using data and samples from 12 international cohorts including more than 10,000 patients with more than 200,000 data points in total. The vision is to develop three tests to predict progression of disease, and to guide treatment with Rifaximin and Albumin.

Aleksander Krag explained the rationale for using Albumin as intervention in decompensation of cirrhosis. Albumin is a well-established, safe and tolerable drug in decompensated cirrhosis with ample evidence for efficacy which is also recommended by the EASL guidelines. However, there is contradicting evidence for long-term administration, and the suggested number-needed-to-treat (NNT) based on the ANSWER trial is rather high (NNT=24). This calls for a more targeted approach with the aim of a lower NNT: MICROB-PREDICT aims to develop a point-of-care device which predicts on a per-patient basis a) who is at risk for progression of disease and b) who will benefit from use of Albumin.

Christophe Junot explained the MICROB-PREDICT approach and route from biomarker discovery performed by research laboratories till the development of a point-of-care test for clinical use. Any of such tests will need to detect complex molecular signatures. Depending on the nature of the biomarker and the size of molecules to be detected, different techniques can be used.

Arben Merkoçi showed briefly the concept of lateral flow sensors (nanoparticle-based devices), which offer the advantage of high sensitivity of the detection system, cost efficiency, easy-to-use by end-users and the possibility of a connection to smart-phone applications for data interpretation. Detecting small molecules, perhaps with low concentrations, is more challenging and can be done with electrical-

based sensors. These are more sensitive and can also be connected to a smartphone application for data interpretation.

Aleksander Krag explained the design and some details of the ALB-trial. The aim of the randomized, placebo-controlled, double-blinded trial in patients with decompensated cirrhosis is to validate the new biomarker as a predictor for disease progression and good versus bad response to Albumin defined by certain cut-off values. Stratification will be based on biomarker response. Intervention will be human albumin infusions. Cumulative number of liver-related events will be outcomes.

Aleksander Krag talked about different options for predictive biomarker study designs and explained our approach with stratification into high expected effect vs. low expected effect, then randomisation into Albumin / Placebo groups. All of this should translate into a lower NNT.

[There was some discussion of the study design presented up to here, which is summarized below.]

Inclusion and exclusion criteria were outlined which have been defined by leading hepatologists in the field. The suggested primary endpoint is cumulative number of liver-related events, incl. a new decompensation episode, liver transplantation, TIPS or death. As statistical approach recurrent event analysis was chosen.

3. Topics discussed

Topic 1 – **Biomarker development: Discovery process, validation process and utilization of the biomarker in a clinical setting.**

A high-level review of the current state of the biomarker discovery process and the potential biosensor techniques (lateral-flow assays, inkjet-based technologies) were presented. It was emphasized that the discovery process will have multiple samples sources (stool, blood and urine) and that discovery cohort amongst others consisted of samples from randomized controlled trials testing human albumin infusions (ANSWER and ATTIRE studies). It was envisioned that the biomarker will provide a dichotomous response, namely, likely versus non-likely to benefit from human albumin infusions. From the clinical study part of the presentation, it was emphasized in which scenarios the investigators believed the predictive biomarker was validated.

In the light of the information provided and the discussion held the following key points were outlined by the experts:

- It was suggested to work with a large database to be retrospectively validated with potential to detect biomarkers. This is an acceptable strategy but cannot be assessed in more detail lacking information on the selection process. Retrospective evaluation is an acceptable approach as preliminary exercise for identification of biomarkers. We don`t have any a-priori concerns.
- Utilization of biomarkers in the clinic is acceptable. The work hypothesis (a subgroup of patients can be identified based on micro-biome based markers that stronger benefit from Albumin treatment, in comparison to the broad population) is valuable but needs to be discussed again later in relation to the qualification process.
- Emphasized that it is important to make a clear separation of what is a discovery process and what is a validation process in the project.
- Argued that in Scenario 2 from the clinical study presentation (Slide 20, meeting presentation) patients in both strata appear to benefit from treatment despite the predictive value of the marker, which would need to be considered in regulatory decision making (and the way future patients are informed about the expected treatment benefit).

- Stated that the relationship between treatment response and predictive biomarker level (or, e.g. combination of markers as a linear predictor) most likely would turn out as a continuum and that is why thresholds and cut-offs should be carefully chosen during marker development.

  ⇨ **MICROB-PREDICT**: There is always a continuum of features. In the middle field economic aspects will become relevant.

- Asks how far we are in the process of selection/finding the biomarker. It could have important implications from a regulatory perspective due to the new IVDR (Regulation EU 2017/746) in the EU coming into force by the 26th of May 2022. With the timeline of the EU consortium in mind, it will fall under the new IVDR. The consortium should bear in mind for the development of their assay whether it could be considered to meet the definition of a companion diagnostic (see Regulatory pathway & requirements discussion).

- Asked why it was mentioned that our methods can be used by consumers. Offered to send more details about regulatory requirements.

  ⇨ **MICROB-PREDICT:** The printers to be used shall be consumer printers. Printing of ink-based nanoparticles is still challenging and not feasible yet for mass fabrication – only for research use. Once we have all parameters, we will role out for larger use and mass production. This would be done by a company.

MICROB-PREDICT:

- How detailed should the biomarker identification process be outlined in the request for scientific advice or regulatory approval?

  ⇨ ITF: Indeed, you will have to present more information and details than today. Maybe a full protocol, at least a protocol outline or synopsis, the methodology of validation, a description of the entire biomarker discovery process (incl. early discovery, identification process and biomarker candidates) and outline which of your methods will fall under the new IVDR.

Topic 2 - Regulatory pathway and requirements under the new in-vitro diagnostic regulation (IVDR): Companion diagnostics considerations, requirements of performance data needed, criteria of approval and relevant regulatory authorities.

Questions were posed regarding the regulatory framework governing both the biomarker discovery process together with the clinical validation study. The applicant presented study design options for predictive biomarkers validation studies with reference to the FDA BEST resource (Slide 18, meeting presentation). Lastly the question was posed whether the biomarker would qualify as a companion diagnostic.

In the light of the information provided and the discussion held the following key points were outlined by the experts:

- ITF highlighted the requirements for clinical performance evaluation and clinical evidence (IVDR Art 56) and the requirement to notify the relevant competent authority, even when using only left-over samples from previous studies (Art. 57 and 58 IVDR). This could have implications for the development / validation process of the biomarker and the analytical performance evaluation.

  For the interventional performance study an important corrigendum (Official Journal of the European Union L.117 of 5 May 2017) has been published concerning recital 66 of the IVDR. There, it is stated that the rules on performance studies should be in line with well-established international guidance in the field, such as the international standard ISO 20916 on clinical performance studies using specimens from human subjects, currently under investigation. The

standard ISO 20916 defines good study practice for the planning, design, conduct, recording and reporting of clinical performance studies carried out to assess the clinical performance and safety of in vitro diagnostic (IVD) medical devices for regulatory purposes. It specifies and expands many of the requirements of the IVDR.

Based on the intended use, as described in the provided document (Annex 1) and the discussion, the IVD may fall under the formal definition of a companion diagnostics (CDX) as provided in Article 2 of the IVDR, For further general information and on companion diagnostics, we recommend the following links and literature:

- https://www.ema.europa.eu/en/human-regulatory/overview/medical-devices
- https://www.ema.europa.eu/en/documents/scientific-guideline/concept-paper-predictive-biomarker-based-assay-development-context-drug-development-lifecycle_en.pdf
- https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-methodological-issues-associated-pharmacogenomic-biomarkers-relation-clinical_en.pdf

  It is recognised that some of the described principles may apply to non-genomic biomarkers in the context of drug development
- Colombage and Lee: Companion diagnostics: Requirments under the new IVD Regulations (Regulatory Rapporteur – Vol 15, No.4, April 2018
- Pum, J.: A practical guide to validation and verification of analytical methods in the clinical laboratory (Advances in Clinical Chemistry, Vol 90, 2019, pages 215-281

- ITF suggested that our product – as one of the options for development – may be used as a companion diagnostic if it is essential for defining patients' eligibility to a specific treatment with a medicinal product or to identify patients at a higher risk of developing an adverse reaction.
- ITF stated that the consortium has an intervention, based on a biomarker response, that may be linked to a medicinal product. Therefore, the consortium may want to approach the biomarker development including the process and documentation to meet the requirements of a companion diagnostic. As of 26 May 2022, if a diagnostic test is developed, a separate application for a performance study will be required in addition to any clinical trial authorisation for the investigational medicinal product or where an authorised medicinal product is used outside of its approved indication. ITF encourages the consortium to reach out to the relevant competent authority where the clinical studies/performance study is planned to ensure that the right documentation is available for timely regulatory approval. Article 57 and 58, Annex I, Annex II IVDR and several standards need to be considered. ITF explains that more guidance from EMA on this aspect may be available in the close future. Also advises the consortium to look into the application documents of other in-vitro diagnostics to identify analytical and clinical requirements.

MICROB-PREDICT:

Albumin is already in use for a broader spectrum of indications according to the core SmPC[1]. It is unlikely that the albumin owners would support this. Is it necessary to file two applications, one for the medicinal product and one for the biomarker, although we are interested in the biomarker only? Do we need to apply in all countries where our study is performed?

⇨ **ITF:** Points out that if it is used in a new context, e.g. with a new indication, then a new clinical trial application needs to be filed. For a new IVD, an application for a performance

[1] https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-core-summary-product-characteristics-human-albumin-solution-revision-3_en.pdf

study needs to be submitted to the respective competent authorities. Advises to look at other IVDs to get a feeling for what is needed. Suggests early exploratory discussions with the relevant national competent authorities. Reminds us that under the new IVDR smartphone applications are relevant for the process and will be considered from a regulatory standpoint.

More specifically, the applicant states in the Annex1- document, that one of the aims of the project is "to design three new tests as easy-to-use tools and point-of-care, smartphone-connected nanobiosensors" (page 3). Moreover, "An App (on Android and iOS) which reads and quantifies a single test format and allows generating new calibration profiles will be developed. The app, by means of a QR code attached on the strips, will be able to detect which analysis is being performed, quantify the data and show the results in a manner that the user will understand, even without medical or biochemical knowledge" (page34).

Based on these functions, the software may fulfil the definition of an In vitro diagnostic medical device (or an accessory to the IVD) according to article 2(2) of the regulation 2017/746. Therefore, the specific elements important for software development should be considered. The following documents might be important to consider:

- MDCG 2019-11: Guidance on Qualification and Classification of Software in Regulation (EU) 2017/745 – MDR and Regulation (EU) 2017/746 – IVDR: https://ec.europa.eu/health/sites/health/files/md_topics-interest/docs/md_mdcg_2019_11_guidance_en.pdf

- MDCG 2019-16: Guidance on Cybersecurity for medical devices: https://ec.europa.eu/health/sites/health/files/md_sector/docs/md_cybersecurity_en.pdf
  The primary purpose of this document is to provide manufacturers with guidance on how to fulfil all the relevant essential requirements of Annex I to the MDR and IVDR with regard to cybersecurity.

- MDCG 2020-1: Guidance on clinical evaluation (MDR) / performance evaluation (IVDR) of medical device software. https://ec.europa.eu/health/sites/health/files/md_sector/docs/md_mdcg_2020_1_guidance_clinic_eva_md_software_en.pdf

It is very important to clarify the intended use of the software at an early stage and it is advised to start the dialogue with regulatory authorities with respect to the particular development to ensure that all applicable regulations are considered.

Topic 3 - Study design: Clinical trial (Alb-Trial) to evaluate a predictive biomarker of human albumin treatment response

The applicant presents the study design of the clinical validation study (Alb-Trial). Stresses the consideration given to the study design bearing in mind the intervention (human albumin), the proposed predictive biomarker and the rationale of long-term human albumin administration with the ANSWER trial as an exemplar. Envisions a study design with two strata and four groups where patients with both high expected effect and low expected effect will be randomized to treatment.

In the light of the information provided and the discussion held the following key points were outlined by the experts:

- Points towards the issue that we probably can expect to see some effect in the low expected effect stratum. Raises the question of when number-needed-to-treat (NNT) is sufficiently low to say that treatment is effective in this group of patients. This also extends to the ethical consideration and questions regarding the false negative patients i.e. those the predictive biomarker will misclassify as not likely to benefit, but who will in fact see an effect of the intervention. Asked whether this will have consequences or an influence on the trial conduct (e.g. influence on patient dosing) and outcomes/endpoints, i.e. what we need to achieve in the end in terms of predictive properties and NNT.

    ⇨ **MICROB-PREDICT:** All biomarkers will be associated with false-negative results. This is true for all clinical tools. Consequences for the treatment regime will depend on the resources in the countries. Approaches need to be taken that help patients best also considering health economics factors.

ITF:

- The ethical question may arise with respect to the dichotomous stratification. Also questions the threshold for clinical relevance of the difference between strata (i.e. not merely the statistical difference between strata). Asks what will be the criteria for telling us whether the biomarker is successful or not? *(the Applicant believes that the assumptions of the sample size calculation for the study also constitute a clinically relevant difference, Study Protocol version 1.0, p. 39).*

    ⇨ **MICROB-PREDICT:** We don't know how the biomarker will perform. In the end we will have two groups: unlikely to benefit/likely to benefit. We think we have chosen the best design to tell us, of course with certain limitations. Biology is continuous, and we need to set cut-offs. Surely, there will be false-positives, too.

    ⇨ **MICROB-PREDICT:** In our presentation, we have not shown results from other studies, which were negative. The presented NNT is our best guess. The ANSWER study showed a benefit of survival, but with a high NNT. There are good reasons for not administering Albumin to all patients: Albumin is human, we need donors. It is very expensive. It may cause complications, e.g. variceal bleeding (see ANSWER-Trial). The decision whom to give it is not easy and that is why we need to personalize the clinical decision-making for administration of Albumin.

ITF:

- Questions the dosing rationale and regimen for the participants in the study. Lower doses were used in the ANSWER trial than what is planned for the clinical validation study "Alb-Trial" and we also chose a 3-month trial compared to the 18-month ANSWER trial. Careful evaluation of all available data is needed (e.g. adverse effects). A deviation from the dosing regimen of other positive studies should be explained in detail. *(the Applicant points the attention to the Study Protocol version 1.0, p. 30, where dosing regimen and rationale is elaborated in detail summarizing the available evidence).*

    ⇨ **MICROB-PREDICT:** Our design is based on opinions of many experts from within and outside our consortium. We think that we have optimized the treatment scheme compared to the ANSWER trial, but we will take this well into account when finalising the protocol.

    ⇨ **MICROB-PREDICT:** Even in the ANSWER trial there is a clear treatment target value after one month. Our 3-month outcome showed clearly that a higher dose is beneficial, and this is backed up by ANSWER data on the albumin-level target after one month. We see that we will need to outline this better in the rationale and summarize better the data that backs up our approach.

ITF:

- States that the applicants should bear in mind that the regulatory approval of the final product (i.e. predictive biomarker) and indication (or "context of use"-statement) will depend on the population investigated in the validation study (i.e. only patients with ascites, Child-Pugh B/C and/or MELD >15). Does not want to suggest that the chosen population is inadequate. The influence of the underlying disease seems to be minor, however, consistency of subgroups will remain to be demonstrated, or else justified in appropriate manner. The question is whether this also applies to decompensation and cholestatic related decompensated liver cirrhosis or whether these patients are different. Indicates that inclusion of patients with different aetiologies of liver disease is encouraged and the underlying aetiology should be well documented. The assumption that patients are similar could be acceptable, however it is always good to have a wide variety of patients included.

Topic 4 - Primary endpoint: Evaluation of clinical efficacy of the predictive biomarker

The applicant presents the proposed composite primary outcome consisting of new decompensation episodes, liver transplantation (also censoring), TIPS (also censoring) and finally death. The applicants have chosen a recurrent event analysis to gauge the effectiveness of the predictive biomarker and effect of the intervention not merely constrained to the first event, as seen in time-to-event analysis.

In the light of the information provided and the discussion held the following key points were outlined by the experts:

ITF:

- Points out that a derogation from the one endpoint of the ANSWER trial (mortality) and use of composite endpoints with different meaningfulness in the Alb-trial could be problematic and needs to be well justified. Would like to see more support for the kidney injury endpoint as part of the new decompensation episodes endpoint. Argues that infections in general (i.e. non-SBP infections) could be problematic as part of the primary endpoint as this is no established outcome in late liver disease. Argues that the use of TIPS as part of the primary endpoint could raise concerns since the decision to apply the TIPS procedure is not standardized across countries (e.g. when there is bleeding, ascites, treatment resistance, etc.) and this could contribute uncertainty. TIPS could also provoke deterioration of hepatic encephalopathy, so TIPS as an endpoint should be questioned. In general, some endpoints need more clear justification. *(The applicant points towards the study protocol version 1.0, p. 21, where referral is made to the LIVERHOPE consortiums position paper on endpoints and design of clinical trials in decompensated cirrhosis).*

- Mentions that the recurrent event approach could be challenging from a statistical point of view, since one event could trigger later events. Also raises the question of patients in the placebo arms receiving the intervention (human albumin) as part of the standard medical treatment for a decompensating event, and how this is addressed in the statistical analyses. With the use of time-to-event analysis these hurdles are avoided. Also thinks that a drop-out rate of 8% is in the lower boundary of what can be expected in this group of patients.

- States that the choice of the primary endpoint (i.e. recurrent event, time-to-event or any other method) should first and foremost be clinically relevant. Questions how deterioration is considered

in the statistical analyses and remarks that this probably depends on the state of the development of the markers. Argues that time-to-event could be another viable statistical approach. However, issues arise with competing risks in the form of liver transplantation for example. Suggests thinking about how to predict decompensation events, if e.g., a patient has received TIPS or needs to be deferred to liver transplantation. More generally, the choice of the primary estimand should clearly reflect the scientific question of interest, strategies handling intercurrent events (e.g. treatment discontinuation, application of TIPS procedure) should be justified and pre-defined and the methods to predict outcome values following such events following withdrawal from the study should be detailed (including sensitivity analyses addressing the robustness of the results with respect to assumption made in the analysis). Provided a recent EMA position on recurrent event analysis to the applicants along the final Brief Meeting Report:

https://www.ema.europa.eu/en/documents/other/qualification-opinion-clinically-interpretable-treatment-effect-measures-based-recurrent-event_en.pdf

- During the discussion also agrees that recurrent event analysis probably yields more insights into the total healthcare burden. However, urges the applicant to carefully consider the primary endpoint and choose a suitable statistical analysis depending on this. Proposes that recurrent event could be an important secondary endpoint.
-

Open questions

- Standards: how far should we go into good laboratory practice regulations?

ITF:

Standards in general and especially harmonized standards are a very important tool to demonstrate conformity with the General Safety and Performance Requirements of a respective legislation. Recital 20 of the IVDR states very precisely: "To recognise the important role of standardisation in the field of in vitro diagnostic medical devices, compliance with harmonised standards as defined in Regulation (EU) No 1025/2012 of the European Parliament and of the Council should be a means for manufacturers to demonstrate conformity with the general safety and performance requirements and other legal requirements, such as those relating to quality and risk management, laid down in this Regulation".

As mentioned before, especially the international standard ISO 20916 on clinical performance studies using specimens from human subjects has a pivotal role for manufacturer.

For further information we would recommend the following GHTF documents focussing on the role of standards in the conformity assessment process:

- http://www.imdrf.org/docs/ghtf/archived/sg1/technical-docs/ghtf-sg1-n063-2011-summary-technical-documentation-ivd-safety-conformity-110317.pdf

  In this document we would like to highlight the principles of the EP checklist.

- This document provides an overview about the role of standards in the assessment of medical devices:

  http://www.imdrf.org/docs/ghtf/archived/sg1/technical-docs/ghtf-sg1-n044-guidance-device-assessment-061003.pdf

- How do we go on from here? What is the process? What are next steps?

ITF:

It is important to clarify the developmental process for companion diagnostics. We recommend contacting the innovation offices of the national competent authorities for further guidance and information.

Moreover, EMA provides developers advice on the most appropriate way to generate robust evidence on a novel methodology and its intention to be validated. Information on novel methodologies qualification procedure can be found here:

https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-advice-protocol-assistance/qualification-novel-methodologies-medicine-development-0

A question-and answer document with key points to consider for the successful qualification of digital technology-based methodologies intended to support the authorisation of medicines is available for applicants:

https://www.ema.europa.eu/documents/other/questions-answers-qualification-digital-technology-based-methodologies-support-approval-medicinal_en.pdf

Alb-Trial

A randomized multicentre, double-blinded and placebo-controlled, trial of human albumin in the treatment of decompensated cirrhosis guided by the Microb-Predict biomarker

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Deputy Principal Investigator
Professor Jonel Trabicka

Sponsor
OUH / EF-CLIF

Supported by
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Tool Revision History

Version Number: 0.1
Version Date (DD/MM/YYYY): 18/04/2018
Summary of Revisions Made:

- Version of the protocol approved through the Microb-Predict Grant Agreement

Version Number: 0.2
Version Date: 02/06/2019
Summary of Revisions Made:

- **Doses**: the dosing regimen of human albumin and placebo has been changed from every 10 day to every 7 day ± 72 hours (Section 4.1)
- **Blinding of study infusion intervention**: Grifols will provide a placebo to the human albumin infusion for the control arm(s) (Section 4.2)
- **Primary endpoint**: has been a revised based on discussion at the 1st Microb-Predict General Assembly. Altered from MELD score change to a reduction in the cumulative number of disease-related decompensation episodes (Section 1.1 and Section 5.3)
- **Protocol**: layout changed to be based on the NCCIH clinical intervention study protocol template version 1.1 of June 2018.

Version Number: 0.3
Version Date: 26/07/2019
Summary of Revisions Made:

- Added EU logo to front page
- Added information on study sites. UKB role is shifted to Johann Wolfgang Goethe-Universität Frankfurt am Main (GUF).
- A first preliminary draft on section 2 (BACKGROUND / RATIONALE) together with section 3 (STUDY DESIGN)
- Cross-checked with most of the SPIRIT 2013 Checklist.

Version Number: 0.4
Version Date: 27/09/2019
Summary of Revisions Made:

- Revised **exclusion criteria** based on comments from Steering Committee Meeting in Copenhagen 11th of September 2019
- Infections (as part of the **primary outcome**) will be defined by Fernandez et al (64)
- Analysis of cost/effectiveness ratio and health economic evaluation has been included as **secondary outcomes**

Version Number: 0.5
Version Date: 29/11/2019
Summary of Revisions Made:

- Revised **inclusion and exclusion criteria** based on comments from Clinical Trial Management Committee meeting at AASLD in Boston, November 2019
- **Age criterium** changed
- Revised list of **infections**.
- Newly added **secondary outcomes** (ICU, serum albumin changes and adverse events)
- **Dosing regimen** back to every 10th day ± 3 days
- **Blinding** of outcome assessors to serum albumin levels
- Local **GCP monitoring** at each study site

Version Number: 0.6
Version Date: 30/12/2019
Summary of Revisions Made:

- A new **sample size calculation** based on the **negative binomial model**.
- Corrected dates of **First patient first visit and last patient last visit**
- Updated figures of **study design** (Figure 2), **event model** (Figure 3) and **study period** (Figure 7)

Version Number: 0.7
Version Date: 26/03/2020
Summary of Revisions Made:

- **Revised in- and exclusion criteria** based on discussions at the 2nd General Assembly of Microb-Predict in Frankfurt January 2020.

Version Number: 0.8
Version Date: 22/05/2020
Summary of Revisions Made:

- Corrected method for blinding the intervention. No iron added to the placebo product but instead opaque bags and infusion lines.
- Defined the eCRF for the study (RedCap)
- Updated figure 2
- Corrections to QoL questionnaires

Version Number: 0.9
Version Date: 03/08/2020
Summary of Revisions Made:

- Draft deleted from header
- Correction of study inception and completion according to MS36 and MS38 in Grant Agreement (Figure 7 and text)
- Revised **role** of the **DSMC**
- Defined **stopping rule** for the **DSMC**
- Added definitions of **hepatic encephalopathy** and **ACLF** in appendices

Version Number: 1.0
Version Date: 12/01/2021
Summary of Revisions Made:

- Minor adjustments to **in- and exclusions criteria** together with **endpoint definitions** according to LIVERHOPE endpoint article (https://doi.org/10.1016/j.jhep.2020.08.009)

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LIST OF ABBREVATIONS

ACLF	Acute-on-Chronic Liver Failure
AE	Adverse Event
AKI	Acute kidney injury
ANCOVA	Analysis of Covariance
CLDQ	Chronic Liver Disease Questionnaire
CTMC	Clinical Trial Monitoring Committee
DSMC	Data and Safety Monitoring Committee
EASL	European Association for the Study of the Liver
eCRF	Electronic Case Report Form
EF-CLIF	European Foundation for the Study of Chronic Liver Failure
EQ-5D	5-Dimension Self-Report Questionnaire
ESLD	End-stage Liver Disease
FCRB	Fundacio Privada Clinic per a la Recerca Biomedica
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GLP	Good Laboratory Practice
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GUF	Johann Wolfgang Goethe-Universität Frankfurt am Main
HA	Human Albumin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HPF	High power field
HRS	Hepatorenal syndrome
ICER	Incremental cost-effectiveness ratio
ICH	International Conference on Harmonisation
IEC	Independent ethics committee
IL	Interleukin
INF-γ	Interferon gamma
IRB	Institutional review board
ITT	Intention-to-treat
KCL	King's College London
KUL	Katholieke Universiteit Leuven
LUMC	Academisch Ziekenhuis Leiden
LVP	Large-volume paracentesis
MELD	Model for End-Stage Liver Disease
NO	Nitrogen oxide
NYHA	New York Heart Association
OUH	Odense Universitetshospital

PAMP	Pathogen-Associated Molecular Pattern
PI	Principal investigator
PP	Per protocol
PPR	Pattern Recognition Receptor
QALY	Quality-Adjust Life Years
RBC	Red blood cell
RCT	Randomized Controlled Trial
ROS	Reactive Oxygen Species
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Spontaneous bacterial peritonitis
SF-36	Short Form 36
SIV	Site initiation visit
SMT	Standard Medical Treatment
SOP	Standard operation procedure
TIPS	Transjugular intrahepatic portosystemic shunt
TNF	Tumour Necrosis Factor
UCL	University College London
UNIDEB	Debreceni Egyetem
WP	Work Package

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PRÉCIS

Study title	**Alb-Trial** *A randomized multicentre, double-blinded and placebo-controlled, clinical trial of human albumin in the treatment of decompensated cirrhosis guided by the Microb-Predict biomarker*
Objectives	**The overall objectives are to predict treatment response to human albumin and to reduce mortality and morbidity in decompensated cirrhosis.** **Primary outcome** Cumulative number of liver-related clinical outcomes (variceal bleeding, ascites, spontaneous bacterial peritonitis, infection requiring hospitalization, acute kidney injury/hepatorenal syndrome - acute kidney injury and overt hepatic encephalopathy) with death, liver transplantation and TIPS as counting and censoring events **Secondary outcomes** 6-months survival The number of acute-on-chronic liver failure Patients' quality of life Time to first hospital admission (in days) Number of hospital admissions Days spent on hospitalization (in days) Number of intensive care unit admissions Length of intensive care unit admissions (in days) Number of large volume paracentesis Analysis of the cost/effectiveness ratio Health economic evaluation Changes in serum albumin levels Number of treatment-related adverse events Number of treatment-related serious adverse events Signatures associated with a poor prognosis as defined by the Microb-Predict biomarker
Study design	Randomized, double-blinded, placebo-controlled, investigator-initiated, multicentre, strata allocated trial
Intervention	Active infusion group: Human Albumin Grifols® 20% covered in opaque infusion bags and lines + Standard Medical Treatment (SMT) Placebo infusion group: Placebo (0.9% NaCl) covered in opaque infusion bags and lines + SMT

	Intervention will be given every 10th day ± 72 hours for 6 months	
Strata allocation	Based on the anticipated effect of albumin infusions (high- vs. low expected effect) according to the Microb-Predict biomarker.	
Duration	**Enrolment period**	22 months
	Subject participation (follow-up)	6 months
Sample size	Using the biomarker to predict the response to albumin **220** (sample size) patients will be stratified into two groups. Participants from both groups will be randomized to albumin + SMT or placebo + SMT. The approximate number of patients in each stratum is hence **55**.	
Population	Patients with decompensated cirrhosis (Child Pugh class B/C and/or MELD score ≥ 15)	
Inclusion- and exclusion criteria	**Inclusion criteria** - Decompensated liver cirrhosis defined as Child Pugh class B or C and/or MELD ≥ 15 - Clinical and/or ultrasound evidenced ascites - Age ≥ 18 years - At least five days since resolution of a decompensation event or any condition requiring hospitalisation. **Exclusion criteria** - Patients with acute or subacute liver failure without underlying cirrhosis - Patients with cirrhosis who develop decompensation in the postoperative period following partial hepatectomy - Refractory ascites as defined by the International Ascites Club - Existing TIPS - Portal vein thrombosis - Full stratification group - Severe alcoholic hepatitis (Glasgow Alcoholic Hepatitis Score > 11) - ≥ Child Pugh C13 - Current, planned or previous treatment with direct antiviral agents for HCV in the last six months - Contraindications for human albumin infusion (pulmonary oedema, hypersensitivity etc.) - Evidence of current malignancy expect for non-melanocytic skin cancer and hepatocellular carcinoma within BCLC-0 or BCLC-A - Presence or history of severe extra-hepatic diseases (e.g., chronic renal failure requiring hemodialysis, severe heart disease (NYHA > II); severe chronic pulmonary disease (GOLD Score ≥ C), severe neurological and psychiatric disorders, pulmonary arterial hypertension)	

	- HIV positive or other condition associated with and/or requiring immunosuppression - Previous liver or other transplantation - Pregnancy - Breastfeeding - Patients who decline to participate or who cannot provide prior written informed consent and when there is documented evidence that the patient has no legal surrogate decision maker and it appears unlikely that the patient will regain consciousness or sufficient ability to provide delayed informed consent - Physician's denial (investigator considers that the patient will not adhere to the study protocol scheduled, e.g. in case of heavy drinking)

1. STUDY PURPOSE AND OUTCOMES

The overall objective of the study is to validate biomarkers to predict treatment response to albumin. The biomarkers will be developed under the Microb-Predict project and tested in a randomized, double-blinded, placebo-controlled, investigator-initiated, strata allocated trial with patients enrolled from eight European study sites. The aim is to provide a clinical tool for personalized treatment with albumin that can detect high-risk patients who are likely to benefit from the treatment. Thus, this study will thereby accelerate the development of biomarkers to be ready for implementation in clinical practice and enable personalized medicine in the realm of decompensated cirrhosis.

1.1 PRIMARY OUTCOME

Cumulative number of liver-related clinical outcomes (variceal bleeding, ascites, spontaneous bacterial peritonitis, infection requiring hospitalization, acute kidney injury/hepatorenal syndrome acute kidney injury and overt hepatic encephalopathy) with death, liver transplantation and TIPS as counting and censoring events

1.2 SECONDARY OUTCOMES

- 6-months survival
- The number of acute-on-chronic liver failure
- Patients' quality of life
- Time to first hospital admission (in days)
- Number of hospital admissions
- Days spent on hospitalization (in days)
- Number of intensive care admissions
- Length of intensive care admissions (in days)
- Number of large volume paracentesis
- Analysis of the cost/effectiveness ratio
- Health economic evaluation
- Changes in serum albumin levels
- Number of treatment-related adverse events
- Number of treatment-related serious adverse events
- Signatures associated with a poor prognosis as defined by the Microb-Predict biomarker

2. BACKGROUND AND RATIONALE

2.1 EPIDEMIOLOGY

Liver cirrhosis is becoming an increasing burden for society (1). The global death toll of cirrhosis was in 2013 1.2 million and an additionally 750,000 died due to liver cancer (2). This is placing liver diseases as one of the leading causes of death worldwide (3). Cirrhosis is not just a key societal challenge for developing countries. In Europe cirrhosis accounts for around 170,000 deaths yearly summing up to 1.8% of all deaths (4).
The most common causes of cirrhosis such as alcohol, obesity and viral hepatitis B and C infections continue to prevail in various degrees and regions of Europe, while some territories experience unprecedented increases in mortality rates as compared to other disease-areas such as cardiovascular and pulmonary (5,6).
The burden of cirrhosis and its complications is also an issue from a health economic perspective. In the United States the cost per patient-per-month for hepatitis C infected patients more than double (from $1870 to $4931) when cirrhosis progress from compensated to decompensated cirrhosis, where majority of the cost for decompensated patients are due to hospitalization (7). The total cost of acute-on-chronic liver failure (ACLF) and cirrhosis are around $1.6 billion and $9.8 billion, respectively surpassing the mean cost per hospitalization for pneumonia, congestive heart disease, septicaemia etc. (2). Figures in Europe can be presumed to be in a similar range where annual health-care cost in the UK has been estimated to be £9120 per decompensated cirrhotic patient (8).
This increasing burden for society is urging for clinical studies considering novel methods aimed at improving prognosis, personalize treatment and hereby reduce the cost of care for patients with cirrhosis.

2.2 PATHOPHYSIOLOGY IN DECOMPENSATED CIRRHOSIS

Cirrhosis is a progressive disease and the end-stage of all known chronic liver diseases. It is characterized by a pro-inflammatory milieu with activated hepatic stellate cells leading to a fibrotic response with proliferation of connective tissue replacing the hepatic cells (9). This tissue remodeling can go unnoticed for decades and the first stage in the natural history of cirrhosis is hence defined from (I) presence of regenerating nodules of hepatocytes and (II) presence of fibrosis, until the first major complication- also known as the compensated stage (10).

Transition from compensated to decompensated cirrhosis happens in the order of 5% to 7% per year (11) and marks a pivotal change from an organ-specific condition to a systemic dysfunction with the potential of multi-organ failure (12).
The progression to decompensated cirrhosis comes with substantial costs for both the individual and society as a whole. From the first decompensation episode the prognosis for survival decrease to 3-5 years (13) and the disease progression is characterized by a markedly decrease in quality of life (14).

The most common complications of cirrhosis include the development of ascites, variceal hemorrhage and hepatic encephalopathy, which defines progression to the decompensated stage of cirrhosis (13).
The pathophysiology behind decompensated cirrhosis is only partly understood and involves several factors. Common hallmarks are increased hepatic venous resistance due to fibrosis leading to portal hypertension and altered hemodynamics leading to effective hypovolemia (15,16). In recent years an altered gut microbiome and dysfunction in the intestinal barrier, often referred to as

the leaky gut, have also been identified to play key roles in disease progression (17–19) (Figure 1). Studies have shown that the leaky gut causes an increased bacterial translocation in decompensated cirrhosis (20,21). The leaky gut hereby causes an imbalance in the gut-liver axis which may lead to increased portal venous pressure and systemic inflammation (22,23)
(24). In a study of the association between disease progression and altered gut microbiome with 219 cirrhotic patients (ranging from compensated outpatients to decompensated inpatients) and 25 age-matched controls it was shown that the degree of dysbiosis was significantly associated with the progression of cirrhosis (25). Other studies have confirmed the role of the microbiota in relation to cirrhosis together with ACLF and its complications in a similar fashion (26,27). Hence, the gut microbiota may carry information that can utilized to predict treatment response and in the guidance of managing cirrhosis (13).

ACLF is a syndrome distinct from acute decompensation of cirrhosis but related to chronic liver disease. More than 13 different definitions of ACLF exist in the current context of acute liver failure in chronic liver disease (13). However, the CANONIC study by the EASL-CLIF consortium where 1,343 hospitalized patients in eight European countries were evaluated, led to the diagnostic criteria's defined by (I) acute decompensation of cirrhosis, (II) presence of organ failure and (III) a high probability of short-term 28-day mortality (28). This diagnostic definition is widely used in Europe and validated in both Asia and North American cohorts to assess patients with decompensated cirrhosis (29–31).
Compared to acute decompensation without ACLF, the addition of ACLF marks a remarkable deterioration in the prognosis. The CANONIC study assessed the 28-day mortality for patients with decompensated cirrhosis and found that the mortality increased from 1.9% to 32.8% with the presence of ACLF (28). Later, similar mortality rates were reported from other geographical regions, stressing the severity of the syndrome (30,32).
However, even though many precipitating events are known (e.g. active alcoholism, bacterial infections, reactivation of chronic HBV) up to 40% of the patients developing ACLF showed no sign of any triggering event (13,28). The findings are striking since it hinders the clinicians' ability to identify patients at risk of developing ACLF and hereby early intervention. At current the only curative therapy in ACLF is liver transplantation and therefore patients with decompensated cirrhotic are in a substantial risk of developing ACLF for which treatment options are scarce, hospitalization costs are high, and survival-probability is low. Early detection and guided therapies to prevent progression to ALCF in in decompensation cirrhosis are urgently needed (33).

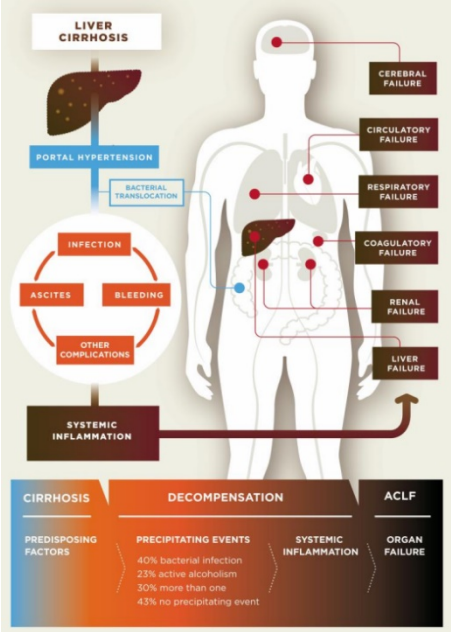


Figure 1 - The pathogenesis of decompensated cirrhosis and acute-on-chronic liver failure

2.3 HUMAN ALBUMIN AND ITS POTENTIAL

Albumin is quantitatively the most abundant protein in the human circulation. Most known is albumin for its plasma expanding effects where it accounts for 70-75% of the oncotic pressure (34) but the plasma protein has as a wide range of other effects. First of all is the molecule an important carrier of hydrophobic molecules such as metals, fatty acids, metabolites and drugs (34). This ensures a timely and necessary degradation and transport of both endogenous and exogenous substances. Secondly is albumin also an important part of the immune system and its immunomodulatory role has only lately truly been acknowledged (35). Albumin has been shown to be an important carrier of prostaglandin E2 which serves as an immunosuppressive agent and which is elevated in acutely decompensated cirrhotic patients (36,37). Furthermore, is the antioxidant effect of the protein due to the large number of reduced sulfhydryl groups important in order to neutralize reactive oxygen species (ROS) from oxidative stress as seen in systemic inflammation (38).

Since albumin is synthesized in the liver, damage to the hepatocytes, as seen in chronic liver disease, impacts both the concentration and function of the protein (35,39). At current, European Association for the Study of the Liver (EASL) guidelines recommend albumin infusions in the management of complication to cirrhosis related to hemodynamic alterations including (1) large-volume paracentesis (LVP), (2) spontaneous bacterial peritonitis (SBP) and (3) hepatorenal syndrome (HRS) (40,41).
However, recently several studies have looked into the long-term administration of human albumin as a prophylaxis to prevent complications of cirrhosis and improve survival (34).
Romanelli et al (42) treated 100 patients with first-onset ascites who were randomized to receive diuretics and albumin (25 grams per week the first year and 25 grams per second week hereafter) or solely diuretics. Results showed that the group treated with albumin had a survival benefit and decreased probability of recurrence of ascites (42) . However, other studies have shown conflicting results with regards to the ability to prevent complications and improve survival rates (43–47).

The largest study performed to assess the effects of long-term administration of albumin in decompensated cirrhosis is the ANSWER study, an open-label RCT performed at 33 Italian hospitals and a total of 440 patients with cirrhosis and uncomplicated ascites (48).
The study found that long-term albumin infusions increased 18-month survival and yielded other positive benefits such as increased time to paracentesis, increased time-to-diagnosis of refractory ascites and an incremental cost-effectiveness ratio (ICER) of the intervention of €21,265 per quality-adjusted life year (QALY) (48). However, methodological issues impede the possibility to draw definite conclusions with regards to the efficacy and cost-effectiveness of long-term administration of human albumin. First of all were the open-label design of the study associated with an inherent bias where the need for large-volume paracentesis and the intensity of medical supervision might have been affected (49). Another limitation was the economic evaluation where the ICER calculation did not account for the indirect costs related to the intervention. This leads to a scenario where the ICER figure might have been underestimated.
More research into the effects of long-term administration of albumin is hence needed and biomarkers to detect subjects who are likely to benefit from treatment would improve cost-effectiveness and reduce overtreatment.

2.4 -OMICS TECHNOLOGIES FOR BIOMARKER DISCOVERY IN CIRRHOSIS

New sequencing technologies such as 16S and mass spectrometry have revolutionized the rate at which new molecule and metabolites are being discovered in the blood, microbiota, urine and saliva (50). All the information gathered from these new technologies with regards to the genome, proteome or metabolome is known as -omics. This opens op for the possibility of reversing the trajectory in which potential biomarker candidates are discovered going from a triangular towards a rectangular strategy (50).
In recent decades the role of the microbiota and dysbiosis in relation to cirrhosis and other disease areas are starting to be appreciated (51–53). Several collaborative efforts such as the MetaHIT and Human Microbiome Project have paved the avenue for novel studies into the gut microbiome and its role in disease (54,55). As mentioned previously, the role of the microbiome is presumably central in the pathogenesis of cirrhosis and transition from a decompensated state to systemic inflammation with ACLF and organ failure as a consequence (13,56,57). Hence, a microbiome biomarker could yield new insights into the role of the gut microbiota and help identifying patients at risk of decompensation and progression to ACLF. Such a tool could be valuable in a clinical setting since existing models to predict survival such as the MELD score do not capture the role of the gut microbiota (58).
Studies into the metabolome of both humans and rodents have shown its role in the development of cardiovascular disease and insulin resistance (59,60). Studies have furthermore hinted at the phenomena of an intestinal microbiota variation in humans that is stratified named enterotypes, which are neither nation- nor continent-specific (61). Such findings linked with the known role of the gut microbiota in other disease areas points towards a potential for a biomarker that can predict the effect of therapies, such as albumin, in cirrhosis (53,62,63).

3. STUDY DESIGN

3.1 GLOBAL STUDY DESIGN

Alb-Trial is an European, multicentre, investigator-initiated, biomarker-stratified randomized, double-blinded and placebo-controlled trial (Figure 2). The goal is to compare the effect of long-term administration of human albumin in a cohort of decompensated cirrhotic patients stratified by their expected treatment response to human albumin. Stratification will happen based on the Microb-Predict biomarker and after assignment to an expected effect group the patients will be randomized 1:1 to the treatment group. Hereby four strata will arise from the stratification and randomization (Figure 2).

3.2 UNIT OF ASSIGNMENT: MICROB-PREDICT BIOMARKER

On inclusion, the biological material for the biomarker stratification is obtained. The biological material is then shipped to **GUF** for analysis. Based on the biomarker, participant will be allocated into one of the two strata (high- or low expected response to treatment) (Figure 6).

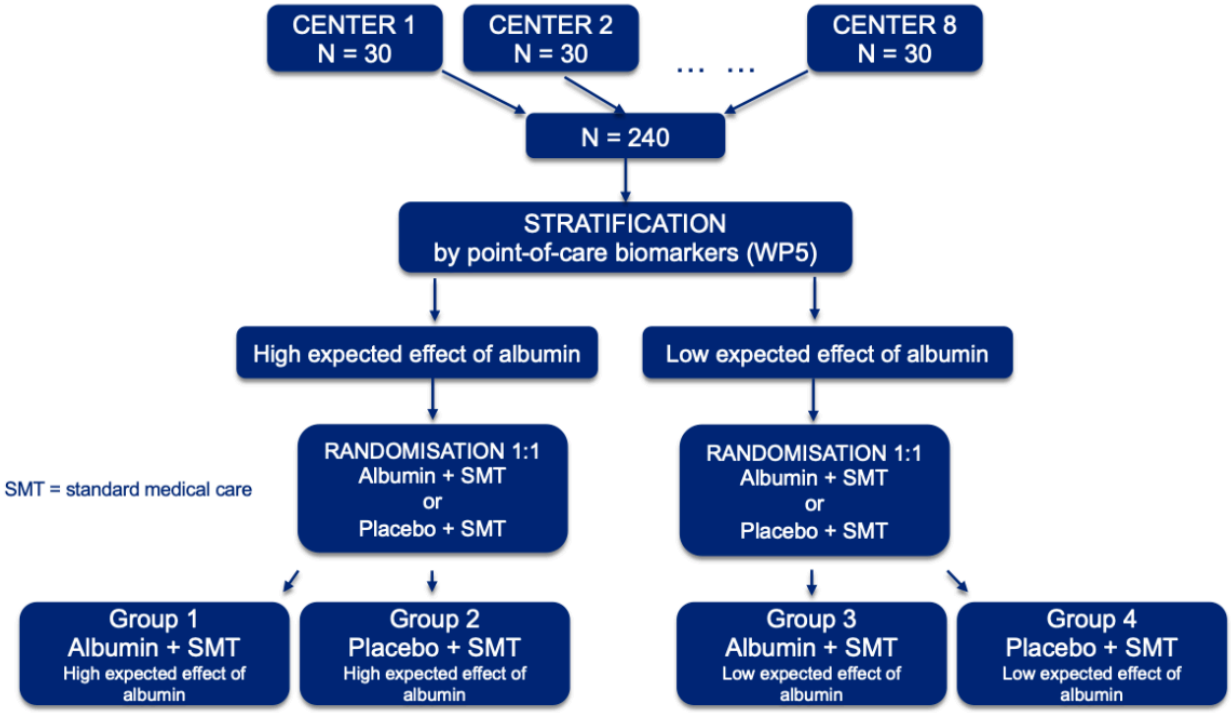


Figure 2 - Study design and allocation process. The duration for each patient is 6 months. SMT = standard medical treatment, HA = human albumin, WP = work package

3.3 OUTCOMES

In order to evaluate the efficacy of the biomarker and human albumin treatment the outcomes are defined as below.

3.3.1 PRIMARY OUTCOME

Cumulative number of liver-related clinical outcomes

Treatment effect will be assessed using recurrent event count of the following events (Figure 3)

- Decompensation episodes
- Death
- Transjugular intrahepatic portosystemic shunt (also censoring)
- Liver transplantation (also censoring)

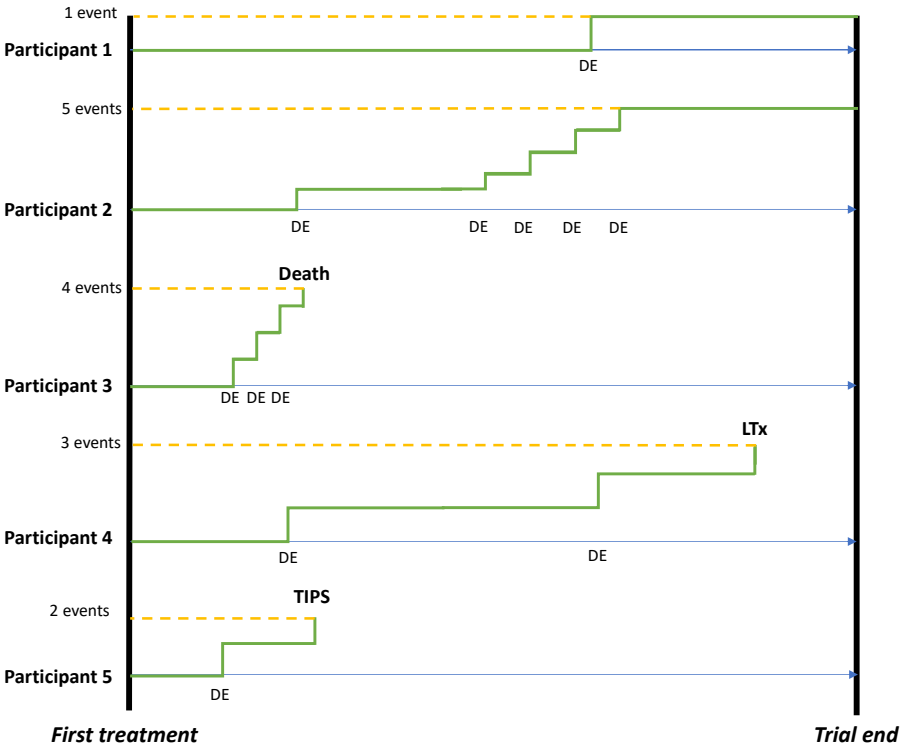


Figure 3 – Example of calculating recurrent events in five scenarios. DE = decompensation events, LTx = liver transplantation, TIPS = transjugular intrahepatic shunt

Decompensation episodes is as defined by EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis (40).

- Variceal bleeding
  Any gastro-oesophageal variceal haemorrhage where the bleeding is confirmed endoscopic

- Ascites
  Any ascites requiring large-volume paracentesis will be regarded as an event.

- Spontaneous bacterial peritonitis
  Bacterial infection of the ascitic fluid with neutrophils $\geq 250/mm^3$ determined by microscopy or flow cytometry together with absence of an evident intraabdominal surgically treatable source.

- Infection requiring hospitalization
  Defined by Fernandez et al (64) and/or Sola et al (65)will be regarded as events in the following cases.

  - Urinary infection: at least one among:

  - Fever >38° C
  - Urgency
  - Frequency
  - Dysuria
  - Suprapubic tenderness
  - Worsening of consciousness

    AND
  - Positive urine culture (>10^5 CFU per cc of urine)

    OR

    At least two among:
  - Fever >38° C
  - Urgency
  - Frequency
  - Dysuria
  - Suprapubic tenderness
  - Worsening of consciousness

    AND

    At least one among:
  - Pyruria (urine specimen with ≥ 10 white blood cells/mm^3)
  - Positive dipstick for leucocytes and/or nitrates

- Spontaneous bacteraemia: one positive blood cultures showing a noncommon skin contaminant OR at least two positive blood cultures showing a common skincontaminant (e.g. coagulase negative staphylococcus, micrococcus, diphtheroid, bacillus species etc.) drawn from separate sites and/or on separate occasions
- Secondary bacteraemia: catheter-related infection (positive blood and catheter cultures) or bacteraemia occurring within 24 hours after an invasive procedure
- Pneumonia: chest x-ray findings (new, or progressive pulmonary infiltrate, consolidation or cavitation)

  AND
  - At least one sign/symptom (new onset of purulent sputum or change in character of sputum; new onset of cough, dyspnea or tachypnea, rales or bronchial breath sounds; worsening of gas exchange)

    AND
  - At least one among:
    - Fever ≥ 38° C
    - Leucocytosis (>12,000/mm^3) or leucopenia (<4,000/mm^3)
    - Altered mental status
- Cellulitis: clinical signs of infection associated with swelling, erythema, heat and tenderness in the skin
- Cholangitis: cholestasis, compatible symptoms (right upper quadrant pain and/or jaundice) and radiological data of biliary obstruction
- Spontaneous bacterial empyema: polymorphonuclear cell count in pleural fluid ≥250/mm^3

- Secondary peritonitis: polymorphonuclear cell count in ascitic fluid ≥250/mm³ and evidence of an intraabdominal source of infection demonstrated by abdominal CT or surgery
- Suspected bacterial infection: presence of signs of infection (fever and leucocytosis requiring antibiotic therapy) without any identifiable source

- Acute kidney injury (AKI)
  AKI stage ≥ 1B according to EASL clinical practice guideline for the management of decompensated cirrhosis (40) (Appendix I)

  OR

  Hepatorenal syndrome – acute kidney injury (HRS-AKI) defined as

  - Diagnosis of AKI
  - No response after two consecutive days of diuretic withdrawal and plasma volume expansion with albumin (1 g pr kg of bodyweight)
  - Absence of shock
  - No current or recent use of nephrotoxic drugs (NSAIDs, aminoglycosides, iodinated contrast medias etc.)
  - No macroscopic signs of structural kidney injury, defined as:
    - Absence of proteinuria (>500 mg/day)
    - Absence of microhaematuria (>50 RBCs per high power field)
    - Normal findings on renal ultrasonography

- Overt hepatic encephalopathy
  Hepatic encephalopathy grade II-IV according to the West Haven Criteria (66) (Appendix II)

3.3.2 SECONDARY OUTCOMES

6-months survival
All-cause and liver-related deaths will be regarded as events in this regard.

The number of acute-on-chronic liver failure
ACLF will be defined according to the definition used in the CANONIC study (28) (Appendix III).

Patients' Quality of Life
Will be measured according to the latest chronic liver disease questionnaire (CLDQ), Short Form 36 (SF-36) and utility index EuroQol Group 5-Dimension Self-Report Questionnaire (EQ-5D).

Time to first hospital admission (in days)
Defined from time of inclusion into the study and until the first admission as registered in medical records.

Number of hospital admissions
Defined as the number of independent admissions to the hospital.

Days spent on hospitalization (in days)
Will be defined as the cumulative number of days spent in hospital from admission to discharge.

Number of intensive care unit admissions
Defined as the number of independent admissions to the ICU.

Length of intensive care unit admission (in days)
Defined as the length of stay in the ICU denoted in days.

Number of large volume paracentesis
Will be defined as the cumulative number of large volume paracentesis performed.

Analysis of the cost/effectiveness ratio
The cost-effectiveness ratio will be analysed in a study-wide setting and study-site (nation) specific manner. It will be calculated according to the ICER:QALY ratio accounting for the direct costs. The QALY based on patients QoL questionnaires.

Health economic evaluation
A broader economic analysis in both a study-wide and study-site (nation) specific manner. The health economic evaluation will account for both the direct and indirect costs incurred in relation to the intervention.

Changes in serum albumin levels
Evaluated in grams per liter at the end of the first month and end of treatment

Number of treatment-related adverse events
Will count as defined in section 7.1.1

Number of treatment-related serious adverse events
Will count as defined in section 7.1.2

Signatures associated with a poor prognosis as defined by the Microb-Predict biomarker

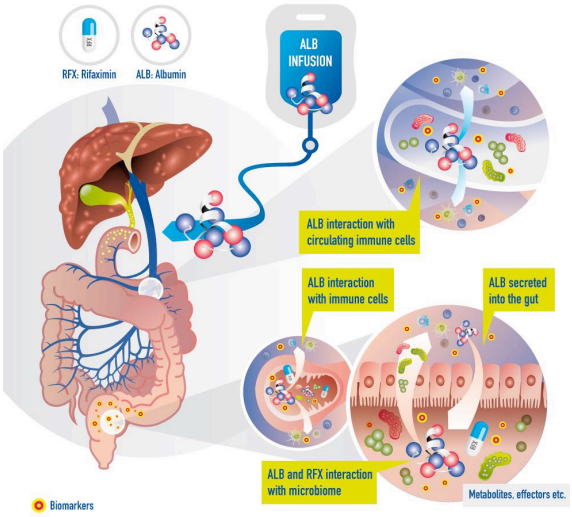


Figure 4 - Interactions of albumin and potential targets of the biomarker

3.4 STUDY POPULATION

The population of the study are decompensated cirrhotic patients with either a MELD score ≥ 15 or Child Pugh Class B or C. This population has a high short-term mortality (58,67) and hence is believed to be a group potentially benefitting from long-term albumin administration in a cost-effective manner. The gender ratio of the trial subjects is expected to reflect the ratio of patients with advanced cirrhosis as described in the CANONIC study with a slight overweight of men (63-65%) (28).

A total of 440 potential participants is expected to be screened during the recruitment period of the study. It is expected that around 55% of all screened patients will fulfil the in- and exclusion criteria and will be willing to participate (Section 3.8).

However, from the total pool of 440 potential participants 240 subjects are expected to be eligible to participate in the Alb-Trial study and with an expected drop-out rate of 8%, 220 are believed to complete the study. Randomization and stratum allocation are described in Section 3.8.

3.5 STUDY SITES

The study is performed in out-patient clinics at eight clinical study sites across seven European countries (see figure 5). All study sites are university clinics with a high volume of patients and all experienced in conducting randomized clinical trials. Each centre treats a minimum of 200 cirrhotic patients per year and of those a minimum of 45 that fulfil the inclusion criteria. All centres are fully committed to the recruitment of patients to the study. Study procedures are outlined in Section 6.

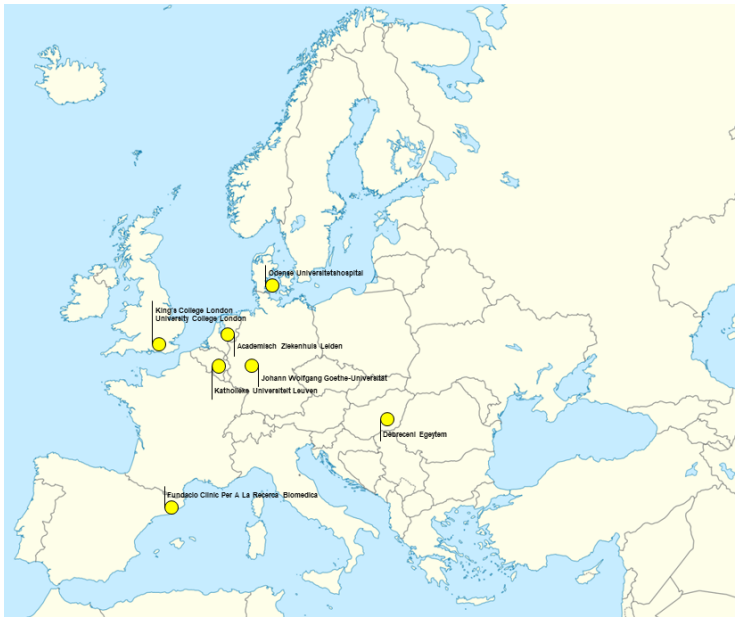


Figure 5 - Location of the Alb-Trial clinical study sites

With 30 patients included from each site within 22 months an average of 1.4 patient(s) must be recruited per site per month (see Table 1). This figure is expected to be feasible. However, as a first contingency measure, competitive recruitment between the eight centres will be imposed when a centre has reached 20 patients or after 10 months. If this does not suffice, the contingency measure of second choice will be to contract with a third party. Such third parties would be chosen among EF-CLIF associated clinical centres in European countries that already have a contributing centre. This criterion for selection of potential third parties will ensure academic quality together with clinical integrity.

Site	OUH	UNIDEB	GUF	LUMC	KCL	UCL	KUL	FCRB
Patients per month	5	4	4	4	5	5	5	5
Expected recruitment per month	3	2	2	2	3	3	3	3

Table 1 – Overview of estimated patients per month, who fulfil the inclusion criteria and the expected recruitment from each study site.

3.6 TREATMENT OF PRE-RANDOMIZATION LOSSES

All patients evaluated for inclusion will be entered in a screening log. If a patient is not enrolled, the screening log will include reason of exclusion. No subject will be re-enrolled after withdrawal. It is expected that screening of 440 patients are needed to reach the sample size.

3.7 SAMPLE SIZE

The effective sample size of the study is 220 patients. When accounting for a drop-out rate of 8% a total of 240 patients will be included in the study. See the sample size calculation in Section 9.2.

3.8 RANDOMIZATION, TREATMENT ASSIGNMENT & BLINDING

Patients fulfilling the inclusion and exclusion criteria outlined in Section 4 will have to sign an informed consent form and provide the biological sample needed for the biomarker-stratification. The biological sample will be analysed centrally at **GUF** according to the developed biomarker(s). If the patient is applicable to a stratum of either high- or low expected effect of human albumin the patient will be assigned to this group. This assignment is double-blinded, where neither the patient nor the study site investigator is aware of which group the patient is allocated to.

After enrolment in a stratum of expected effect the patient is randomly assigned to a treatment group. The allocation to treatment groups will happen centrally by **EF-CLIF** and be performed online after inclusion and screening of the patient. Treatment assignment will happen in a 1:1 ratio from a computer-generated randomization list in permuted blocks of two and four. Given that there will be a stratum of high- and low expected effect together with two different treatment groups, a total of four strata will arise (Figure 6). The treatment group will also be double-blinded to patients and study site investigators by opaque bags and black infusion lines.
To ensure blinding of investigators at site none of the investigators at study sites will have access to albumin measured in blood samples.

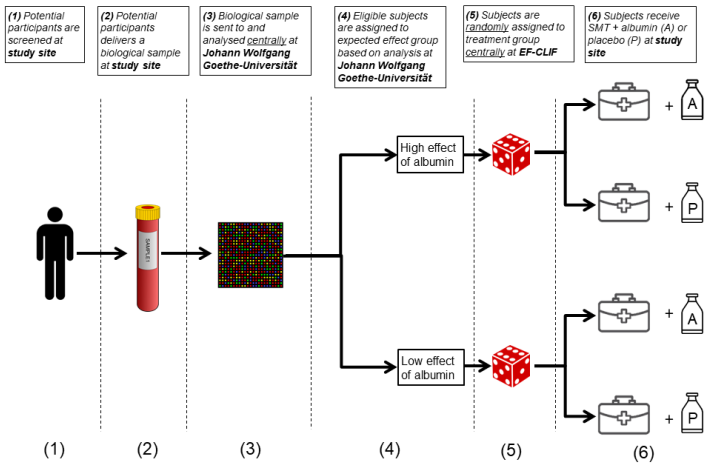


Figure 6 - Treatment assignment. Investigators and patients at study sites are double-blinded to both expected effect group and treatment

3.9 RECRUITMENT AND FOLLOW-UP PERIOD

The duration of the recruitment period is expected to be 22 months. As the follow-up period of the study for each patient is six months the study will be finalized 28 months after expected inclusion of the first patient. Hence the inclusion of the first patient will be in June 2022 and last patient last visit will be in September 2024 (Figure 7).

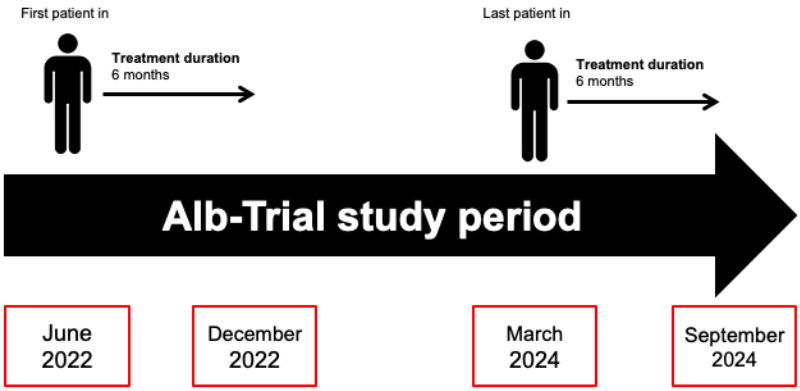


Figure 7 - Alb-Trial study period

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 INCLUSION CRITERIA

- Decompensated liver cirrhosis defined as Child Pugh class B or C and/or MELD ≥ 15
- Clinical and/or ultrasound evidenced ascites
- Age ≥ 18 years
- At least five days since resolution of a decompensation event or any condition requiring hospitalisation.

4.2 EXCLUSION CRITERIA

- Patients with acute or subacute liver failure without underlying cirrhosis
- Patients with cirrhosis who develop decompensation in the postoperative period following partial hepatectomy
- Refractory ascites
- Existing TIPS
- Portal vein thrombosis
- Full stratification group
- Severe alcoholic hepatitis (Glasgow Alcoholic Hepatitis Score > 11)
- ≥ Child Pugh C13
- Current, planned or previous treatment with direct antiviral agents for HCV in the last six months
- Contraindications for human albumin infusion (pulmonary oedema, hypersensitivity etc.)
- Evidence of current malignancy expect for non-melanocytic skin cancer and hepatocellular carcinoma within BCLC-0 or BCLC-A
- Presence or history of severe extra-hepatic diseases (e.g., chronic renal failure requiring hemodialysis, severe heart disease (NYHA > II); severe chronic pulmonary disease (GOLD Score ≥ C), severe neurological and psychiatric disorders, pulmonary arterial hypertension)
- HIV positive or other condition associated with and/or requiring immunosuppression
- Previous liver or other transplantation
- Pregnancy
- Breastfeeding
- Patients who decline to participate or who cannot provide prior written informed consent and when there is documented evidence that the patient has no legal surrogate decision maker and it

appears unlikely that the patient will regain consciousness or sufficient ability to provide delayed informed consent
- Physician's denial (investigator considers that the patient will not adhere to the study protocol scheduled, e.g. in case of heavy drinking)

4.3 STUDY ENROLLMENT PROCEDURE

Participants will primarily be recruited from the study sites where eligible patients are recruited from inpatient wards, outpatient clinics and the intensive care units. However, recruitment will also be conducted through local/national patient organizations and from the uptake areas of the study sites.
In short, all patients invited to participate in the Alb-Trial study will receive patient information sheets and informed consent forms containing both general- and specific information about the Alb-Trial clinical study. All information for potential participants will be written in a clear and simple way without unnecessary use of excessive legal references.
Patients that are not able to consent themselves, will have the possibility to consent through a representative following establish procedures. All information will be provided in a language and way that the patient or the representative can fully understand. For those who are not able to read and/or write, consent can be obtained through other medias (video, audio recordings etc.). All patient information will be translated into local languages of the study sites. To ensure that patients have understood the implications of participation and their rights, checklists developed in the Microb-Predict framework will in this way check the free will of potential participants. The consent procedures for the study will follow the informed consent policy developed in the Microb-Predict consortium framework. All consent procedures adhere to The Helsinki Declaration (2013) (Appendix IV).

4.4 WITHDRAWAL CRITERIA

Patients may be withdrawn from the trial by the investigator or terminate their participation prematurely based on:

- Post-consent decision due to inclusion error (determination of ineligibility based on safety or eligibility criteria)
- Physician's judgment following an adverse event post-treatment
- Patient's decision to withdraw consent
- Termination of the trial by a regulatory authority
- Any other reason for withdrawal that the trial physician or patient feels is in the best interest of the patient.

If a patient is withdrawn from the study based on decisions from study staff, the patient will receive an adequate explanation for their withdrawal from the study.

5. INTERVENTION

5.1 BIOMARKER STRATIFICATION OF EXPECTED TREATMENT RESPONSE

All participants will undergo blinded stratification according to the expected response to albumin. The prediction of response will be based on a biomarker, that currently is under development in the Microb-Predict consortium and consequently the final biomarker is not yet ready to use. The

biomarker will be based on measurements of (disease specific) molecules from biological material. The biological material will stem from either salvia, blood, urine or stool. The biomarker could potentially be based on data from the biological material in question together with other traits or the phenotype of the patient. The biomarker will provide a dichotomous output where the patient can have high or low expected effect of human albumin infusions.

In the unlikely event that the biomarker has yet been developed at the inception of the clinical study no stratification will occur according to treatment response (Figure 2). In this case, the clinical trial will run as planned without a pre-randomised biomarker stratification. Instead validation of the biomarker will be done after completion of the study.

5.2 MEDICAL INTERVENTIONS

All participants will receive albumin or placebo in out-patient clinics at the study sites. Infusion of the study drugs will happen every 10th day ± 72 hours. All participants must receive the initial treatment within seven days after the inclusion. Any patient who experience one of the prespecified outcomes after inclusion in the study but prior to randomization will be excluded from the study. The blinding of the medical intervention is described in Section 5.6.

5.2.1 HUMAN ALBUMIN INTERVENTION

The intervention is intravenous infusions with human albumin. The dosing regimen have historically been an unclarified point in long-term administration studies. Doses have ranged from 25 grams every second week to 40 grams a week. The two most recent studies of the effect of long-term human albumin administration have been the ANSWER study (median MELD: 12-13) and the MACHT study (median MELD:16-17). The two studies used different dosing regimens (Table 2) and came to ambiguous conclusions with regards to the prevention of complications of cirrhosis (46,48).
Yet, more recent reports from Fernandéz et al (68) describes that doses in the order of 1.5 grams per kilo bodyweight per week (maximum of 100 grams) of human albumin are needed in order to stabilize albumin levels in serum and lower systemic inflammation. Hence, Alb-Trial will follow this dosing regimen of 1.5 g/kg bodyweight and a maximum of 100 grams per patient per infusion. Infusion intervals will be every 10th day ± 72 hours.

Study	**Dosing regimen**	
	Loading dose	Dose hereafter
	Continuous dose	
Gentilini (1999) (43)	25 grams per week	12.5 grams per week
Romanelli (2006) (42)	25 grams per week	12.5 grams per week
Solá (2018) (46)	20 grams per week	
Caraceni (2018) (48)	80 grams per week	40 grams per week
Pascoli (2018) (47)	40 grams per week	
Fernandéz (2019) (68)	100 grams per week	
Alb-Trial	**100 grams per week**	

Table 2 – Human albumin infusions doses in other relevant studies. Note that doses have been transformed into weekly doses to make dosing regimens compatible. Furthermore, a 70 kilogram reference was used for studies where infusion volumes were based on bodyweight.

5.2.1.1 HUMAN ALBUMIN PREPARATION

Grifols S.A. 20% human albumin solutions (approved medical product) will be provided for the study.

5.2.1.2 HUMAN ALBUMIN STORAGE AND HANDLING

Grifols S.A. 20% albumin vials have to be stored at a temperature of between 2-25ºC. The shelf life is three years when maintained at this temperature. The products should not be frozen. Once the container is open, the contents must be administered immediately. Unused contents must be discarded according to the study SOP. No remaining product in an opened container can be stored in the refrigerator and used at a later time. This product must not be used beyond the expiry date stated on the labelling. The solution is normally clear to slightly opalescent. The product will not be used if the solution is cloudy or has precipitated. The product must be warmed to body temperature before use. Access to the product must be strictly limited and guided by study SOP.

Grifols S.A. 20% human albumin solution supplied for the trial is to be destined exclusively to the study. Any unused material must be returned to the sponsor or at the discretion of the sponsor, may be destroyed at the centre according to study SOPs. A designated person at each study site will keep record of drug utilization according to the study SOP. The sponsor will provide specific forms to be completed by the designated person at the time of dispensation. These forms can be replaced by proprietary forms of the study centre, provided they meet the requirements of the sponsor. Once the forms have been completed, they must be signed and dated by the monitor and by the investigator to confirm their accuracy.

5.2.1.4 PRECAUTIONS OF HUMAN ALBUMIN

Human albumin infusions will be performed at a maximum rate of 50 ml every 15 minutes. Hypersensitivity and allergic reactions are unusual but can occur with human albumin. The signs and symptoms include:

- Urticaria
- Dyspnea
- Wheezing
- Hypotension
- Tachycardia
- Facial reddening
- Palpebral edema

Mild allergic reactions will be controlled with antihistamines and/or cortisone.
In the rare event of anaphylactic manifestations, 0.3-0.5 mL of epinephrine 1:1000 should be administered subcutaneously and repeated in absence of adequate response.
In all instances infusion rates will either be reduced or suspended following study SOP procedures.

A rare but serious adverse event to human albumin infusions is acute pulmonary overload. Symptoms of a pulmonary oedema is (I) a sudden shortness of breath (dyspnoea), (II) sweat, (III) nausea and (IV) anxiety. Scheduled human albumin infusions will not be administered in patients with an establish diagnosis or clinical suspicion of pulmonary oedema and/or lung pneumonia. A suspected acute pulmonary overload will be treated according to the study SOP.

5.2.2 PLACEBO

A placebo drug to human albumin will be used in the study. The placebo will consist of 0.9% NaCl. Infusions of placebo will happen in same intervals as the administration of human albumin. Bags and lines of both human albumin and placebo will furthermore be opaque to ensure an identical visual appearance. Hence, the administration of the placebo will be identical to that of human albumin. All blinding procedures are described in Section 5.6.

5.3 STANDARD MEDICAL TREATMENT

Study sites will provide standard medical treatment of all participants. The standard medical treatment for decompensated cirrhosis will be based on the recommendations and guidelines from relevant scientific societies and regulatory bodies (40,66,69–72). Usual treatments for decompensated cirrhosis are thus allowed. Any current indication that requires human albumin infusions is allowed. Current treatment for co-morbidities will be allowed according to medical judgement. Any medication administered during the study (including blood products) will be recorded centrally in the eCRF.

5.4 ADHERENCE ASSESSMENT

Discontinuation of study treatment for more than four consecutive weeks will exclude the participant from the study. Participants are also excluded if they have not received the initial treatment within seven days from inclusion into the study.

5.5 ADVERSE EVENTS DURING INFUSIONS

Any adverse event, whether serious or not will be detected, recorded and reported according to Safety Assessment section (see section 7.2 and 7.3). In short will any serious and non-serious adverse events detected during the planned intervention infusions be handled according to the study SOPs.

5.6 BLINDING

Both investigators and patients will be blinded to treatment. Grifols S.A. will provide the human albumin and both the active treatment and the placebo drug will be placed in opaque bags and with covered infusion lines. All patients will after stratification be given a central alpha-numeric identification code and hereafter be randomized to treatment centrally at EF-CLIF. Based on the randomization outcome will the study personnel at each site denote the alpha-numeric identification code to the bags of study medication. This medication will be identified by a unique identifier code (UIC) labelled by local hospital pharmacies and will be provided by EF-CLIF to study personnel. Hence, will each bag with a UIC be linked to a patient through the alpha-numeric identification code. Only data managers at EF-CLIF will be unblinded to treatment through the UIC and alpha-numeric identification code link. In the eCRF will the study personnel at each site have the possibility of breaking the blind in case of an SAE during a treatment infusion. As soon as the blinding have been broken will the patient be excluded from the study and only followed hereafter with the patient's own acceptance.
All outcomes will be reviewed prior to statistical analysis and unblinding of serum albumin levels.

6. STUDY PROCEDURES

6.1 SCHEDULE OF EVALUATIONS

	Inclusion visit	Centrally	First visit	Bimonthly visit	Ordinary visits*	Last visit
Written consent	X					
Inclusion/ exclusion criteria	X					
Medical history	X					
Register medications	X					
Biological material for biomarker & biobank	X		X	X		X
Pregnancy test	X					
HIV test	X					
MELD score & Child Pugh Score	X		X	X	X	X
Blood samples	X		X	X	X	X
QoL questionnaires	X					X
Albumin infusion			X	X	X	
Check for decompensation	X		X	X	X	X
Analysis of biomarker†		X				
Allocation to strata†		X				
Randomization to treatment arm‡		X				

Table 3 – Schedule of evaluations. HIV = human immunodeficiency virus; MELD = Model for End-Stage Liver Diesase; QoL = Quality of Life.
** Study visits during the trial include bimonthly visits with blood samples and other visits will be without blood samples. A visit will take place every 10 days ± 72 hours.*
† Analysis of biomarker and allocation to strata will take place at GUF. This is blinded to both patients and investigators.
‡ Randomization to the treatment arms will be performed centrally at EFCLIF. Treatment is blinded to both patients and investigators.

6.2 VISIT SCHEDULE

6.2.1 INCLUSION VISIT

(Performed by a study physician, the following actions and tests will be performed)

- Obtaining of written consent
- Review of inclusion and exclusion criteria

- Medical history
- Register medications
- Biological material for biomarker study is obtained to be sent to GUF (Frankfurt, Germany) for strata allocation and to EF-CLIF (Barcelona, Spain) for distribution into WP5-partners for further biomarker validation; shipments will be organized by EF-CLIF and will be on dry-ice.
- Pregnancy test in all fertile women
- Computation of MELD score and Child Pugh score
- Blood sample collection for determination of baseline values
- QoL questionnaires
- Biological material collected for biobank for future research

6.2.2 CENTRALLY

(Performed at GUF and EF-CLIF)

- Biological material for the biomarker is shipped with expedited transport to GUF
- Patients are allocated into the two strata according to results from the biomarker test performed at GUF; Information of strata-allocation is kept blinded for the investigators
- Patients are by EF-CLIF randomly assigned into the two treatment arms (SMT + HA or SMT + placebo). Information about treatment is blinded to the investigator and patient.

6.2.3 FIRST STUDY VISIT

(Performed by a study physician)

- Assignment of study nurse
- Infusion of albumin or placebo
- Check for signs of hepatic decompensation
- Biological material for biomarker study is obtained to be sent to GUF (Frankfurt, Germany) for strata allocation and to EF-CLIF (Barcelona, Spain) for distribution into WP5-partners for further biomarker validation; shipments will be organized by EF-CLIF and will be on dry-ice.
- Computation of MELD score and Child Pugh score

6.2.4 BIMONTHLY VISIT

(Performed by a study nurse; Performed after 2 and 4 months)

- Infusion of albumin or placebo
- Check for signs of hepatic decompensation
- Identification of possible adverse events in all treatment arms.
- Routine blood test
- Biological material for biomarker to be sent to EF-CLIF for WP5
- Biological material collected for biobank for future research
- Computation of MELD score and Child Pugh score

6.2.5 ORDINARY TREATMENT VISIT

(Performed by a study nurse; Takes place every 10 days ± 72 hours. Bimonthly visits will take the place of an ordinary visit)

- Infusion of albumin or placebo
- Check for signs of hepatic decompensation

- Identification of possible adverse events.
- Routine blood test
- Computation of MELD score and Child Pugh score

6.2.6 LAST VISIT

(Performed by a study physician; Takes place 6 months after inclusion)

- Computation of MELD score and Child Pugh score
- Quality of life questionnaires
- Routine blood test
- Biological material for biomarker – to be sent to EF-CLIF for WP5
- Biological material collected for biobank for future research

7. SAFETY ASSESSMENT

7.1 DEFINITION AND DESCRIPTION OF ADVERSE EVENTS

Adverse and serious adverse events are defined according to ICH-GCP (73).

7.1.1 DEFINITION OF ADVERSE EVENTS

An adverse event (AE) is defined as any untoward medical occurrence in a patient participating in the trial. An AE does not necessarily have a causal relationship with the infusion of albumin. An adverse event can therefore be an unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of albumin, whether or not it is related to infusion of albumin. This includes worsening of a pre-existing condition or increase in frequency of a pre-existing condition.

7.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS

A serious adverse event (SAE) is any untoward medical occurrence happening to the trial participants that: Results in death, is life-threatening, results in persistent or significant disability/incapacity, is a congenital abnormally or is a suspected transmission of any infectious agent via a medicinal product. Apart from this, any event that requires hospitalization or prolongs an existing hospitalization during the cause of a patient's participation in the study must be reported as an SAE. Other medical events that are not included in the above definitions can be reported as an SAE by the treating physician if he or she considers it appropriate to do so. A SAE does not necessarily have a causal relationship with the infusion of albumin. The final decision on the causal relationship will be taken by the Data and Safety Monitoring (DSMC).

7.1.3 ATTRIBUTION DEFINITIONS

- **Not Related**: An AE that is not related to the use of the drug.
- **Doubtful**: An AE for which an alternative explanation is more likely, eg, concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely.
- **Possible**: An AE that might be due to the use of the drug. An alternative explanation, eg, concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.
- **Probable**: An AE that might be due to the use of the drug. The relationship in time is suggestive (eg, confirmed by dechallenge). An alternative explanation is less likely, eg, concomitant drug(s), concomitant disease(s).
- **Very Likely**: An AE that is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, eg, concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive (eg, it is confirmed by dechallenge and rechallenge).

7.1.4 SEVERITY CRITERIA

An assessment of severity grade will be made using the general categorical descriptors outlined in the WHO Toxicity Grading Scale. The investigator should use clinical judgment in assessing the severity of events not directly experienced by the subject (e.g., laboratory abnormalities).

- **Mild (GRADE 1)**: Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
- **Moderate (GRADE 2)**: Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required
- **Severe (GRADE 3)**: Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible
- **Life-threatening (GRADE 4)**: Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable

7.2 DETECTION AND RECORDING OF ADVERSE EVENTS

The AEs and SAEs will be recorded for all treatment arms on every study visit. The basis for recording adverse events can be clinical observation of the patient, laboratory analyses, spontaneous communication from the patient and through open questioning from the investigator.
All adverse events and serious adverse events will be recorded in the patient's medical record and in the electronic case report format (eCRF) on a special Adverse Events Form. AEs and SAEs will be graded according to their attribution and severity (see Section 7.1.3 and 7.1.4).

In the context of this clinical trial, all incidents clearly related to progression of the disease will not be considered as adverse events. It will not be necessary to include them in the Adverse Events Form part of the eCRF and to the local regulatory authorities but will be recorded since they are considered as the primary endpoint.
Hospitalisations are not considered SAEs when they are due to:

- Elective or previously scheduled treatments for pre-existing clinical conditions that are not correlated with the liver cirrhosis
- Emergency treatment in outpatient clinics because of events that do not satisfy the definition of serious adverse events and do not lead to the admission to in-patient wards

Adverse events will be classified according to severity, depending on the intensity of the event and the likelihood of attribution to albumin infusion will be assessed by the DSMC.

7.3 REPORTING PROCEDURES

All AEs whether serious or non-serious, will be reported to the DSMC, Clinical Trial Management Committee (CTMC) and to the sponsor.
The study site investigator assumes responsibility for appropriate reporting of AEs to the local regulatory authorities. The investigator must report suspected SAEs to the appropriate Ethics Committee that approved the protocol.

8. INTERVENTION DISCONTINUATION

If during intervention infusion a patient present with any signs of an allergic reaction to study drug the infusion will be reduced or suspended temporarily.

If during or prior to an intervention infusion a patient present with symptoms or signs of pulmonary edema the infusion will be suspended, and the intervention will temporarily be discontinued.

If a patient is discontinued from the study, they will be followed throughout their expected duration time in the study. A patient will only be followed after discontinuation with their own permission.

A patient can only be temporarily discontinued from the study drug for up to four weeks in a row. Hereafter they are considered to be excluded from the study.

Each subject has the right of withdrawal from the study at any time. In addition, the investigator may discontinue a subject from the study at any time if the investigator considers it necessary for any reason, including the occurrence of adverse events or non-compliance with the protocol.
If a patient is withdrawn from the study based on decisions from study staff, the patient will receive an adequate explanation for their withdrawal from the study.

Local or national regulatory authorities can at any time terminate the study at any study site.

9. STATISTICAL ANALYSIS PLAN

9.1 GENERAL DESIGN ISSUES

According to the primary outcome of the study it is expected that patients treated with human albumin, and who are in the high expected effect group, will have a significantly lower cumulative number of decompensation episodes compared to the three other groups. Indeed, it may be expected that those treated with human albumin but are in the low expected effect group will experience some effect of the intervention. However, this effect will presumably be significantly lower than that for the high expected effect group.

All efficacy analyses will be carried out as intention-to-treat (ITT) and safety analyses as per-protocol (PP).

A statistical analysis plan (SAP) will be issued and approved prior to the initiation of the study.

9.2 SAMPLE SIZE AND RANDOMIZATION

Assumptions: It is expected that the incidence of events can be estimated by an exponential function. The mean observation time, when accounting for censoring events is assumed to be 5.5 months. An incidence rate ratio of 0.65 is expected based on the arithmetic mean of complication rates from the largest RCT testing albumin in outpatients with decompensated cirrhosis (48).

The sample size calculation is based on an equal size of each treatment arm ($\theta = 1$), a type I error of 5% and a power of 80%. A dispersion parameter (κ) of 0.12 was used to correct for an expected overdispersion from the Poisson distribution to the gamma distribution (negative binomial model). This yielded a total of 55 patients in each stratum. With an expected drop-out rate of 8% a total of 240 patients would be included. Hereby it will be possible to show a significant effect of albumin in the high expected effect group versus the low expected effect group. As shown in the sample size formula below $z_{\alpha/2}$ is the two-sided type I error, z_{β} is the power. V_0 and V_1 is the variance of the parameter β_1 under the null- and alternative hypothesis, respectively. λ_1 and λ_0 is the ratio of the true event rates between two groups.

$$n_0 = \frac{(z_{\alpha/2} \cdot \sqrt{V_0} + z_{\beta} \cdot \sqrt{V_1})^2}{\log\left(\frac{\lambda_1}{\lambda_0}\right)^2}$$

Assuming equal variance under the null- and alternative hypothesis, the sum of the variance under the two hypotheses becomes

$$V_0 = V_1$$

$$V_{0,1} = \frac{1}{\mu_t} \cdot \left(\frac{1}{\lambda_0} + \frac{1}{\theta \cdot \lambda_1}\right) + \frac{(1+\theta) \cdot \kappa}{\theta}$$

Assuming a mean follow-up time, when accounting for censoring, of 5.5 months (μ_t), a true rate of λ_0 and λ_1 of 0.438 and 0.284, respectively and $\theta = 1$ and $\kappa = 0.12$ the variance becomes

$$V_{0,1} = 1.30$$

Plotting the data into the sample size formula, the size of each strata hence becomes

$$n_0 = \frac{(1.96 \cdot \sqrt{1.30} + 0.84 \cdot \sqrt{1.30})^2}{\log(0.65)^2}$$

$$n_0 = 55$$

After enrolment in a stratum of expected effect the patient will be randomly assigned to a treatment group. The assignment to treatment groups will happen centrally at **EF-CLIF** and be performed online after inclusion of the patient. Treatment assignment will happen in a 1:1 ratio from a computer-generated randomization list in permuted block sizes of two and four.

9.3 DEFINITION OF POPULATIONS

Two populations will be defined in this study.
The intention-to-treat (ITT) population is defined as all randomized patients, who fulfilled the inclusion- and exclusion criteria and who received at least one infusion treatment.

The per-protocol (PP) population is defined as all of the ITT population who did not present serious protocol violations, who had at least 80% of the treatment infusions and who were not withdrawn from the study due to non-adherence (interruption of treatment for four weeks or more).
Efficacy and safety analyses will be performed on the ITT population.
Sensitivity analyses on efficacy and safety will be performed in the PP populations to confirm the results from the ITT population.

9.4 INTERIM ANALYSES AND STOPPING RULES

A Data and Safety Monitoring Committee (DSMC) will serve as the interim safety review board (ISRB). The DSMC will compromise of three leading experts within the field of Hepatology and with extensive DSMC experience.
The DSMC will review any issues concerning the safety of the trial participants by reviewing serious adverse events and review the integrity of the data and possible statistical problems, such as selection bias, cross-over, failure to diagnose, failure to treat or failure to follow-up. It will work to a charter to be set up prior to inception of the study. Following trial completion, the DSMC will assist in interpretation of the final results and approve the main trial publication. No interim analyses of the primary outcome are planned during the course of the study. Interim analyses will be performed for adverse- and serious adverse events but only by the DSMC and the data manager unblinded to strata and treatment allocation.

The stopping rule of the DSMC will be based on a sequential probability ratio test where the DSMC can deem to stop the study if the acceptable rate of treatment-related adverse events has been exceeded. For the course of this study $\alpha = 5\%$, $\beta = 15\%$, π_A (acceptable rate of treatment-related adverse events) = 3%, π_0 (baseline rate of treatment-related adverse events) = 1% and e is the number of treatment-related adverse events.

$$n(e) = \frac{\ln(1-\beta) - \ln(\alpha) + e[\ln(1-\pi_A) - \ln(1-\pi_0) - \ln(\pi_A) + \ln(\pi_0)}{\ln(1-\pi_A) - \ln(1-\pi_0)}$$

The role of the statistical stopping rule is not to have a conclusive rule but will serve as guidance for the DSMC into whether the study should be terminated prematurely due to concerns of patients' safety.

9.5 OUTCOMES ASSESMENT

Besides having both investigators and patients at study sites blinded to treatment groups, blinded independent outcome assessor(s) will review and adjudicate the primary and secondary clinical outcomes.

9.5.1 PRIMARY OUTCOME

The ratio of decompensation rates will be based on the negative binomial model. The model accounts for varying variance in between subjects and the effect of early withdrawals. Estimates will be provided with a 95% confidence interval and the SMT + HA with high expected effect will sequentially be tested against the three other groups.

Assessment of decompensation events will happen from first study visit, at ordinary visits, bimonthly visits and the final visit. If a participant does not show up for a visit, the responsible investigator at each study site will contact the participant and make detailed follow-up check for decompensating events. Hence, the outcome assessment every 10th day in relation to the treatment with study drug during participation.

9.5.2 SECONDARY OUTCOMES

Six-months survival, time-to-first hospital admission will be summarized by means of Kaplan-Meier survival estimates and compared between strata with means of the log-rank chi-square test.

Number of LVPs, days spent in hospital, QoL measurements, number of ACLFs, number of hospital admissions, days spent on hospitalization, number of intensive care admissions, length of intensive care admissions, changes in serum albumin levels, number of treatment-related adverse events, number of treatment-related serious adverse events will be assessed as numeric and count data. QoL will be measured by CLDQ, SF-36 and EQ-5D at baseline and at the conclusion of the study for each patient.

Any potential confounders will be identified at baseline characteristics and accounted for in multiple linear regression analysis.

9.6 DATA ANALYSES

Descriptive statistics will be performed for all four strata of patients and for the individual centers. Categorical parameters will be presented by frequencies and proportions. Continuous parameters will be summarized by mean ± standard deviation or median and interquartile range according to distribution.

Demographic data, clinical features, hematological and biochemical data, MELD-Na, Child-Pugh Class, endoscopic findings, diuretics and concomitant medication together with the clinical history and QoL measurements will be summarized at baseline characteristics.

For the primary outcome analysis, a negative binomial model with incidence rates and rate ratios to compare the effect of albumin between strata will be employed.

For the quantitative variables, linear regression with the strata as an interaction term will be used. Within strata, the differences in primary and secondary outcomes between the two treatment arms will be analyzed with Cox proportional hazards ratio, log-rank tests and Fisher's exact test. Student's t-tests will be used for testing between-group differences in normal-distributed quantitative variables, while Wilcoxon rank sum test will be used for non-parametric variables.

All statistical tests will be two-sided and the significance level $P < 0.05$ unless otherwise stated. 95% confidence intervals will be reported.

9.7 MISSING VALUES

Any missing values will not be imputed after the termination of the study. The statistical analysis will be performed in an available data only fashion.

9.8 SUBGROUP ANALYSES

Subgroup analyses will be performed with regards to the following features: study site, complicance/adherence, Child-Pugh score and initial serum albumin.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 COLLECTION AND MANAGEMENT

Data will be filled in prospectively on the electronic case report format (eCRF) RedCap which will be hosted from OUH and managed by a data manager from EF-CLIF. Data manager at EF-CLIF will perform ongoing review of uploaded eCRFs and send data queries to the centres in case of omissions/supposed errors or need for clarification. Data collection and data management will be conducted in compliance with the principles of the Declaration of Helsinki (2013), the principles of Good Clinical Practice (GCP) and follow the study protocols approved by local ethic committees at each site/country.

10.2 SECURITY AND PROTECTION

At each study site, there will be a study coordinator (co-investigator) responsible for data management in accordance with the principles mentioned above. In all cases, the co-investigator will be responsible for keeping records of data collection and experiments undertaken in line with Good Laboratory Practice (GLP). Proper conduct of data collection and quality of data collected will be monitored by staff at each site.
All epidemiological and patient-related data will be coded in such a way that individual persons cannot be identified from the corresponding data (directive 95/46/EC) according to regulations (unique pseudocode identifier). Medical records will be stored at study sites where they were originally obtained. In the eCRF identities will be anonymized, and biomaterial will be coded with the anonymized identifiers and separate database linking the unique pseudocode to the participant's names. No personal data will be stored or be accessible off-site (see further ethics self-assessments per site for all details). To facilitate data sharing, ethical agreements with patients regarding data that will be collected will include a clause for permission to share anonymized data.

10.3 SAMPLE MANAGEMENT

Blood samples for routine biochemistry will be analysed at the local laboratory of each centre. Biological material for the biomarker test in the clinical trial will be shipped to GUF. Biological material collected during the study will be sent to EF-CLIF with expedited transport and from here sent to WP5 partners and to OUH for biobank for future research. These samples will be handled according to the referred procedure and conditions in accordance to the study SOP.

10.4 QUALITY ASSURANCE

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigator and study-site personnel before the study, periodic monitoring visits by the sponsor, and direct transmission of clinical laboratory data from a central laboratory into the sponsor's database. Written instructions (SOPs) will be provided for collection, handling, and storage of samples.

10.4.1 QUALITY CONTROL COMMITTEE

The quality control committee of the study will be the DSMC. The structure of the committee is outlined above (section 9.4) and will ensure that the necessary steps are taken in order to control the quality of the study and the data. A quality management review tool will be developed to track and document quality assurance at all study sites.

10.4.2 TRAINING

Each study site will be open for enrolment of patients after the coordinating site OUH (with a Principal Investigator Team consisting of Aleksander Krag, Mads Israelsen and Nikolaj Torp) has conducted a Site Initiation Visit (SIV). Prior to this SIV, the investigators at each study site will write a local monitoring plan and a training plan. Both plans must be approved by co-investigator and Principal Investigator Team before the SIV. The training plan must describe the required training of study personnel in study-relevant procedures and in GCP. During the SIV, the Principal Investigator Team will train the local investigators and collaborators about the study protocol, requirements, albumin infusions, visits and completion of (eCRF). In order to ensure homogeneity and the same quality standards, the training procedures made by the Principal Investigator Team will be the same at each study site.

10.4.3 MONITORING

A local GCP unit will be in charge of the monitoring throughout the study at each study site. It is the role of each study site to find a suitable local GCP monitor. The goal of the monitoring will be to compare the data entered into the eCRFs with the hospital records (source documents). Direct access to the source documentation (medical records) will be allowed for the purpose of verifying that the data recorded in the eCRF are consistent with the original source data. The monitor expects that, during monitoring visits, the relevant study-site personnel will be available, the source documentation will be accessible, and a suitable environment will be provided for review of study-related documents. The following types of monitoring visits will be conducted.

Post-initiation visit

The first post-initiation visit will be made as soon as possible after enrolment at each study site has begun. This is in order to ensure that the quality standards are upheld through the whole course of the study.

Close-out visit

A close-out visit will be performed before the last patient last visit of each study site. A general overview and a final revision of pending queries will be implemented, ensuring that all data and corresponding source documents are in place before site closure.

Other visits

The sponsor will perform on-site monitoring visits as frequently as necessary. If required by the sponsor, the study sites must provide personnel for an unannounced inspection on a short notice. The number of planned monitoring visits will be described in detail in the monitoring plan, which will be finalized before the conductance of the SIV.

10.5 PROTOCOL CHANGES AND AMENDMENTS

Neither the principal investigator nor the sponsor will modify this protocol without a formal amendment by the sponsor. All protocol amendments must be issued by the sponsor and signed and dated by the principal investigator. Protocol amendments must not be implemented without prior independent ethics committee (IEC) approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the subjects, in which case the amendment must be promptly submitted to the IEC and relevant competent authority.

Documentation of amendment approval by the investigator and IEC must be provided to the sponsor. When the change(s) involves only logistic or administrative aspects of the study the IEC, where required, only needs to be notified.

10.5.1 PROTOCOL DEVIATIONS

During the course of the study, in situations where a deviation from the protocol is unavoidable, the study personnel in attendance will contact the appropriate co-investigator who will contact the Coordination Team. Except in emergency situations, this contact should be made before implementing any departure from the protocol. In all cases, contact with the sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the eCRF and source documents will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it. A protocol deviation tracking log will be developed in order to track any non-adherence to the study protocol. Co-investigators and personnel at each study site will be responsible for updating the log with all relevant deviations. The deviations will be coded according to their deviation type and used for internal quality assurance. Any protocol deviation should also be reported to the local Independent Ethics Committee whenever applicable.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 INDEPENDENT ETHICS COMMITTEE REVIEW

This protocol and the informed consent documents and any subsequent revisions will be reviewed and approved by relevant Independent Ethics Committee (ICH) responsible for the oversight of the study. All aspects of the study will be done under the conduct of good clinical practice (GCP). The study will adhere to all relevant guidance from regulatory bodies, included but not limited to

- ICH. Note for guidance on good clinical practice. CPMP/ICH/135/95, 1996.
- Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use
- Article 1.2(a) of Council Directive 93/42/EEC concerning Medical Devices.
- Article 1.2(b) Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices.
- European Commission Medical Devices: Guidance document (MEDDEV 2.1/6).
- Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data
- National legal regulatory documents as applicable

11.2 INFORMED CONSENT FORMS

The consent procedures for the study will follow the informed consent policy developed in the Microb-Predict consortium framework (see Appendix I).
In short, all patients invited to participate in the Alb-Trial study will receive patient information sheets and informed consent forms containing both general- and specific information about the Alb-Trial clinical study. All information for potential participants will be written in a clear and simple way without unnecessary use of excessive legal references.
Patients that are not able to consent themselves, will have the possibility to consent through a representative following established procedure. All information will be provided in a language and way that the patient or the representative can fully understand. For those who are not able to read and/or write, consent can be obtained through other medias (video, audio recordings etc.). All patient information will be translated into local languages of the study sites. To ensure that patients have understood the implications of participation and their rights, templates and checklists developed in the Microb-Predict framework will in this way check the free will of potential participants.

All consent procedures will from an ethical perspective adhere to the Helsinki Declaration (2013), see Appendix IV.

11.3 PARTICIPANT CONFIDENTIALITY

The informed consent obtained from the patient will include explicit consent for the processing of personal data and for the investigator to allow direct access to his or her original medical records for study-related monitoring and for inspection by the sponsor and the local regulatory authorities. The consent also addresses the transfer of the previously encrypted data to other entities and to other countries within the framework of the study. The collection and processing of personal data from patients enrolled in this study will be limited to those data that are necessary to fulfil the objectives

of the study. Appropriate steps will be taken to ensure confidentiality and compliance with applicable data protection laws and regulations.
Participants' confidentiality will be ensured through the Informed consent policy for human subjects, human biological samples and personal data framework within Microb-Predict (Appendix IV). Any biological material collected throughout the study will be stored in a pseudonymized manner. Transfer of biological material and/or data to partners outside the European Union will be safeguarded by additional contracts between the principal investigator and the entity in question (Appendix IV)
Every study site will ensure that the principles of data protection will be in line with the regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016, the general data protection regulation (GDPR) and any national/local regulation wherever applicable.

11.4 STUDY DISCONTINUATION

The study can at any time be stopped or temporarily paused at study sites if deemed necessary by national/local authorities. A study site can be excluded from the trial if the study site and institution does not live up to its obligations according to the Microb-Predict Consortium Agreement.

12. COMMITTEES

12.1 DATA AND SAFETY COMMITTEE

The DSMC will comprise three experts with DSMC experience, chaired by an experienced DSMC chair. All members must be independent of the trial and of the sponsoring organization. Members will be nominated and agreed on at a Steering Committee meeting in January 2021. The DSMC will review any issues concerning the safety of the trial participants by reviewing serious adverse events, review the integrity of the data and possible statistical problems, such as selection bias, cross-over, failure to diagnose, failure to treat or failure to follow-up. It will work to a charter to be set up before the beginning of the study. Following trial completion, the DSMC will assist in interpretation of the final results and approve the main trial publication.

The DSMC will meet once per year and advise the Steering Committee and the coordinator/WP leader of the clinical trial/WP leader of data management on any critical issues. The exact powers and tasks will be defined by the Steering Committee.

12.2 CLINICAL TRIAL MONITORING COMMITTEE

The CTMC will consist of Microb-Predict scientists that will coordinate and supervise the clinical trials. It will be led by Aleksander Krag (OUH, WP7 leader) and will further consist of Jonel Trebicka (EF-CLIF, coordinator), Maria Papp (UNIDEB, WP1 leader), Minnekke Coenrad (LUMC, WP9 leader), Pere Gines (FCRB) and Paolo Angeli. The tasks of the CTMC comprise setting up and implementing the logistics around all clinical trials, providing support to Microb-Predict members concerning the clinical trial, and finally reviewing and assessing the results of the clinical trials and assessing whether any changes to the study protocol are necessary.

13. PUBLICATION OF RESEARCH FINDINGS

Any positive and negative results will be published in international peer-reviewed journals in open-access format according to the Horizon 2020 grant agreements.

The authors of the Alb-Trial publications will include grant holder of Microb-Predict (Jonel Trebicka), Principle Investigator Team (Nikolaj Christian Torp, Mads Egerod Israelsen, Aleksander Krag), co-investigators and members of the Clinical Trial Monitoring Committee.

Study physicians who actively contributed will be acknowledged in the end of any manuscript. Other study site members will be acknowledged in the appendix of any publication.

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15. APPENDICIES

Appendix I – AKI definitions and stages

	Stage(s)	Definition
AKI	-	Increase in SCr ≥0.3 mg/dL (≥26.5 µmol/L) within 48 hours; or, a percentage increase in SCr ≥50% from baseline which is known, or presumed, to have occurred within the prior 7 days
AKI stages	Stage 1	Increase in sCr ≥0.3 mg/dL (26.5 µmol/L) or an increase in sCr ≥1.5-fold to 2- fold from baseline. - **Stage 1A**: SCr at diagnosis <1.5 mg/dL - **Stage 1B**: SCr at diagnosis > 1.5 mg/dL
	Stage 2	Increase in SCr >2-fold to 3-fold from baseline.
	Stage 3	Increase of SCr >3-fold from baseline or sCr ≥4.0 mg/dL (353.6 µmol/L) with an acute increase ≥0.3 mg/dl (26.5 µmol/L) or initiation of renal replacement therapy
Table definitions adapted from (40). Table layout inspired by (65).		

Appendix II – West-Haven Criteria

West Haven Criteria (66)		
Grade	*Description*	*Severity*
Minimal	Psychometric or neuropsychological alterations of tests exploring psychomotor speed/executive functions or neurophysiological alterations without clinical evidence of mental change.	Covert
Grade I	Trivial lack of awareness Euphoria or anxiety Shortened attention span Impairment of addition or Subtraction Altered sleep rhythm	
Grade II	Lethargy or apathy Disorientation for time Obvious personality change Inappropriate behavior Dyspraxia Asterixis	Overt
Grade III	Somnolence to semi-stupor Responsive to stimuli Confused Gross disorientation Bizarre behavior	
Grade IV	Coma	

Appendix III – ACLF

Acute-on-chronic liver failure, organ failure definitions (28)	
Organ	*Definition*
Liver failure	Serum bilirubin ≥ 12 mg/dL
Kidney failure	Serum creatinine ≥ 2.0 mg/dL or renal replacement therapy
Cerebral failure	West Haven grade III or IV hepatic encephalopathy
Coagulation failure	International normalized ratio > 2.5 and/or platelet count ≤ 20 x 10^9/L
Circulatory failure	Use of vasopressors (e.g. dopamine, dobutamine or terlipressin)
Respiratory failure	Partial pressure of arterial oxygen / FiO_2 ≤ 200 or SpO_2 / FiO_2 ≤ 200

Appendix IV - Informed consent policy for human subjects, human biological material and personal data in Microb-Predict (MS41)

1. Project objective

Decompensation of liver cirrhosis and progression towards acute-on-chronic liver failure (ACLF) causes 1.2 million deaths/year. Microbiome is causally involved in cirrhosis progression and is for drugs the first interaction point with the patients. Drugs can alter the microbiome leading to unwanted effects or even facilitating their effects, but the microbiome metabolizes the drugs, shapes their effects and possibly determines the host response to drugs. As each person carries an individual microbiome, insight in these processes should help stratify or even personalize patient health care and treatment.

The aims of MICROB-PREDICT are 1) to better understand the role of microbiome and the gut-liver-axis interactome with respect to microbiome functionalities, 2) to identify and validate microbiome-based biomarkers and signatures for personalized prediction of decompensation and ACLF, and response to treatment, 3) to design three new tests as easy-to-use tools and point-of-care, smartphone-connected nanobiosensors, and 4) to validate them in a randomized controlled trial. MICROB-PREDICT will assemble existing data and samples from major microbiome initiatives in hepatology (12 international studies, >10,000 patients), and enrich them with holistic and in-depth analysis using cutting-edge multi-omics technologies of host and microbiome from different body sites in samples of >1,000 patients collected in a longitudinal manner with sequential visits and controlling for confounders.

MICROB-PREDICT results will foster more accurate, personalized risk stratification and significant steps towards personalized treatment of decompensated cirrhosis and ACLF. World-leading microbiome specialists, technology leaders and clinical experts make this a programme of scientific excellence; patient organisations (ELPA) and the European Association for the study of the Liver
(EASL) will channel our results into a powerful dissemination, communication and exploitation programme.

2. Introduction

The MICROB-PREDICT research project deploys different interventions in research: clinical trials with patients and healthy volunteers, biomedical research with human biological samples from diverse origins (blood, stool, mucosa, etc.) and collection, transfer and processing of personal data. These different types of research require designing specific research protocols, information and informed consent procedures and templates to assure the protection of rights of participants, while promoting freedom of research at any stage. This policy describes the ethical and legal framework for the planned research interventions, the implications for research participants (patients and healthy volunteers) and obligations for those conducting research: First, we will elaborate on the information that potential study participants should have received in order to be able to decide whether or not to participate ('informed consent'), and the implications this decision may have for their own personal situation. Second, in line with the requirements introduced by the General Data Protection Regulation (GDPR), we will define and summarize the information that should be included in the information process and informed consent procedure when informing participants,

collecting and using human biological samples and personal data for research purposes. Our information and informed consent policy respects also the main ethical and legal guidelines on ethics in research at international and European level and the research integrity that the Consortium should take into account for achieving the goals of the project, while respecting research subjects.

3. Information for potential human subjects in research and informed consent procedures

To promote autonomy of potential participants in research and according to international and European requirements for information and informed consent procedures information about MICROB-PREDICT research interventions should include the following general information: title of the study; clear information, comprehensive for the general population, about the purpose and content of research, the investigators in charge of the research and findings, adding official references of the project. This information should be followed by a brief description informing potential participants about the proposed research intervention for them to decide whether or not to participate. Participants should be aware that the research has been approved and reviewed by the entitled research ethics committee of the center or research institution involved.

Specific information should be provided about the ethical and legal framework applicable integrating international, European, national and local regulations, depending on the type of research (i.e. clinical trial) in a clear and simple way, avoiding excessive legal references. The final goal is to provide potential participants with all necessary tools to make a rational, free, and voluntary decision that is confirmed by the requisite of informed consent. It is neccesary to check that the participant decides to be part of the research process based on voluntary grounds.

The patient information sheet and the informed consent form are part of a process that includes verbal communication and written information that obliges the investigators to provide any information that participants could need at any time of the research cycle:

It is necessary to provide them with full information about the principal investigator: name, surname, phones and contact address to them to contact him/her at any time and to state clearly that they can ask any question.

It must be explained that study participants are free to participate in any research activity and that they may revoke consent at any time without any explanation or justification. It should furthermore be stated that there will be no negative consequences for them and their care (i.e. receiving treatments, if there is the case, etc.) should they change their mind at any time. In this sense the information provided should be adequate for the general population and should tend to avoid therapeutic misconceptions of potential participants.

If participants are not able to consent, representatives should be informed, and their consent be collected following the established procedures. The information should be provided in a language and way that potential participants or their representatives can fully understand. Those who cannot read and/or write can consent through other specific formats (video, audio recordings etc.). In general terms, the information should be adapted to the level of understanding of the potential participant and translated into the different languages of the Consortium. Local and cultural issues related to the patient information sheet and informed consent issues should be integrated and respected.

The principle of respect for human vulnerability and personal integrity should underlie the whole research process meaning that individuals and groups of special vulnerability should be protected, and the personal integrity of such individuals be respected. In light of the Convention on the Rights of
Persons with Disabilities, United Nations (2016), persons with disabilities should be included in the research process, and the research process should include in the research project following the universal design approach[1]. The principle of non- discrimination for any reason including genetic conditions and disabilities shall be respected.

The process of information continues with a general description that should be very brief, with relevant information expressed in clear and understandable terms according to the level of understanding of potential participants. The main content of this patient information sheet and informed consent templates should include what the research is about (indicating and defining the type of research: clinical trial, biomedical research, etc.); the main goal; the methodology and techniques; the study duration; possible benefits, and known and possible risks of the participation in the study. Information on the number of visits should be included, and if there will be complementary tests. Furthermore, inclusion and exclusion criteria and what happens during visits (if any) etc. could be described.

The main goal is to make potential participants aware of any tests and interventions that will be done outside of routine clinical care, and only because of their participation in the study. The principle investigator in charge of the research intervention should provide clear information on the impact expected on participants as individuals, but also to the groups that they could represent. Potential participants must be informed about their responsibilities within the proposed research, e.g., about the procedures and the obligation to inform about any adverse event that could happen to them.

It is important to make potential participants understand that their participation in the study does not necessarily imply a direct benefit to their health or specific condition. In this sense, they should be aware that their contribution could also be for the general advancement of scientific knowledge. Potential participants should be informed about alternative treatments and interventions to the ones proposed, if there are any, that are effective for their situation. In case of doubt, the principle investigator will be available to provide the necessary information. Furthermore, research participants should know that in specific research arms or activities, being a participant does not modify the standard treatment or intervention (i.e. observational studies). Insurance policies, when applicable, should be explained according to regulations in place.

If it is the case, potential participants should be informed about the possibility and consequences of compassionate use in research meaning that under strict conditions, products in development can be made available to groups of patients who have a disease with no satisfactory authorised therapies and who cannot enter clinical trials (source: European Medicines Agency). Partners of the Consortium in charge of the research with compassionate use should consult the rules and procedures of each Member State.

[1] "Universal design" means the design of products, environments, programmes and services to be usable by all people, to the greatest extent possible, without the need for adaptation or specialized design. "Universal design" shall not exclude assistive devices for particular groups of persons with disabilities where this is needed. (Article 2 Convention on the Rights of Persons with Disabilities, United Nations, 2016)

Monetary or in-kind compensations, if any, should be carefully analyzed by the investigators and the research ethics committees in order to ensure that potential participants are not tempted, pressured or induced by them. Equally it should be clearly stated, if no compensation of any kind is offered.
Participants should be aware about the conditions for reimbursement of travel, dietary or other expenses occurring due to their participation in the study. Any kind of compensation offered should not affect or modify the free will or influence the participants unduly in their decision about their study participation.

MICROB-PREDICT research must avoid any kind of conflict of interest and, if any, investigators must declare it and investigators and institutions should avoid research misconduct or any questionable behaviour. Participants should understand that there are different types of conflict of interests, e.g. economic, of a personal or hierarchical nature, etc. At any time, researchers should check and avoid misconceptions of participants regarding the specific intervention proposed.

Finally, MICROB-PREDICT patient information sheets and informed consent processes need to specify in any type of research intervention any other information considered necessary to be explained to potential participants. Any new information regarding the treatment or interventions used in the study that may affect the willingness to participate, or that could be discovered during participation, will be communicated to those affected by the entitled persons (principal investigators) as soon as possible. If participants decide to withdraw their consent to participate in a study, no new data will be added to the database and participants may require the destruction of all identifiable samples previously held to prevent further analysis.

Participants should also know that they can be excluded from the study if the principal investigator and entitled researchers of the study consider it appropriate. Reasons may include, e.g., safety reasons, any adverse event that occurs and is considered related to their participation, or because participants consider that the study is not complying with established procedures. In any case, if the withdrawal of a study participant was decided by any study staff, participants should receive an adequate explanation of the reason for their withdrawal from the study.

To assure and check that potential participants have properly understood the implications of their participation and their rights, the legal and ethics team of MICROB-PREDICT developed templates and checklists, which include questions and answers to provide information in the easiest way and to check the free will of the subject. In this sense, an “experimental bill of rights” has been developed; a catalogue composed by ten issues to include but not to limit the participants’ rights in the research process has been elaborated.

Finally the verbal and written process of information is accompanied by an informed consent template covering all these aspects to be signed by participants, where they agree or not to participate in the proposed research intervention. The specific template should include again general information about the project (title, reference, funding, principal investigator, etc.) and the name and surnames of the potential participant. The informed consent template should clearly state that participants: 1) have read the information template given to them about the research study after being informed of the above mentioned issues; 2) are able to ask questions about the study; 3) received enough information about the study; 4) have spoken with (name of the principle investigator); 5) understood that their participation is voluntary and that they can withdraw from the study at any time and without giving any explanation and with no negative impact on their situation (medical care, etc.)

4. Information about personal data protection: privacy and confidentiality of potential participants in research

A core issue for the MICROB-PREDICT Consortium is protecting privacy, confidentiality, integrity and dignity of patients and healthy volunteers. Protection of personal data linked to human biological samples is of particular relevance considering the multi-omics use of samples.

Each institution as part of MICROB-PREDICT, where research is carried out, is responsible for assuring privacy and personal data protection of participants and for that the data controllers are clearly identified (indicating who is in charge, including address and contact information). The principles of data protection according to Regulation (EU) 2016/679 of the European Parliament and of the

Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data (GDPR) should be followed during all biomedical research: lawfulness, fairness, non-discrimination, transparency, accuracy, necessity, data minimisation, purpose limitation, privacy by design and by default and of course, explicit and legitimate purpose relying on informed consent of the research participants. These principles allow researchers to respect human rights of potential participants in research. In addition, the new figure of Data Protection Officers established by the GDPR, as independent advisors on the issue of data protection in each research institution, is crucial. Likewise, the Data Protection impact assessment introduced by the mentioned regulation to evaluate the impact of the research specifically in the potential subjects to be involved. These are institutional and practical requirements that help to prevent, to protect and to promote the rights and freedom of involved study subjects, including the freedom of research of the research team.

In general terms, as it has been described before in the context of informed consent, participants should be informed in a simple, adequate and clear way about their rights and the procedures established to make their rights effective according to GDPR and other regulations applicable (i.e. National Laws on Data Protection). To protect privacy and personal information of participants, including special categories of data such as health, genetic data, etc. participants should be informed about the applicable techniques: codification, pseudonymisation[2] or anonymisation and the impact of these techniques on their privacy and lives. Participants should be informed that their identity will be protected and no one will have access to this information, unless there is a justification. The aim is to prevent participants from being identified, understanding that investigators and those with a specific permission and access could, if it is justified, connect personal data of participants with i.e. medical records. Only entitled authorities (i.e. medical healthcare), and Research Ethics Committees involved and staff authorized by the study leader, will have access. Any staff in contact with personal data will apply the highest standards of protection of privacy and respecting secrecy and confidentiality as stated in deontological, ethical and legal regulations.

The study protocol should explain if there will be encrypted data transfers to third parties and/or to other countries. This means that there will be no information that can identify the participant directly (such as name and surnames, initials, address, social security number, etc.). If a transfer of encrypted data is made outside the European Union, either to entities related to a research center where the study is conducted, or to service providers or to researchers who collaborate with the principle investigator, participant data will be protected by safeguards such as contracts or other mechanisms established by the data protection authorities (i.e. Privacy Shield).

In addition to the rights already covered by the legislation (access, rectification, cancellation and opposition), participants should be informed that according to GDPR, they can also limit the processing of incorrect data, request a copy or transfer data that they have provided for the study to a third party (data portability). To exercise these rights, or if participants want to know more about confidentiality, they should contact the principal investigator of the study or the Data Protection Officer of the center. Participants also have the right to contact the National Data Protection Agency (National, local authority entitled) if they are not satisfied with the handling of their data.

[2] 'Pseudonymised' means to divide the data from its direct identifiers so that linkage to a person is only possible with additional information that is held separately. The additional information must be kept separately and securely from processed data to ensure non-attribution." Source: ethics self-assessment European Union (version 4th February 2019) http://ec.europa.eu/research/participants/data/ref/h2020/grants_manual/hi/ethics/h2020_hi_ethics-self-assess_en.pdf

Participants should be informed that if they decide to leave the study, no new data will be collected, but data generated for research purposes until that point in time cannot be destroyed. Participants should be informed about the obligations of researchers and promoters to keep the data collected for the study and for how long after its completion. Personal information will only be kept by the entitled research center/institution and by the main investigator for other scientific research purposes if the participant has given his/her consent to do so, according to the ethical and legal framework applicable.

Participants should declare in writing (or adapted to specific format to give consent) that in accordance with the provisions of Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data (GDPR) and the national regulation applicable (if applicable), they have been informed of the existence of a file or treatments of personal data, of the purpose of the collection of their data and the recipients of the information. According to GDPR, participants could consent to the handling of their personal data after the information process to use personal data in the framework of the specific research and to provide a broad consent in order to be used in further research projects that will be part of the same line of research initiated. Then, they can proceed to consent or not (by ticking the box yes or no) and include their signature and the signature of the principle investigator indicating the date. Specific consent should be added about the right of participants to receive information derived from the research that could be relevant for their health.

5. Information to donors of human samples from biological origin and personal data associated

MICROB-PREDICT research interventions obtain, use, store and share biological samples of human origin and personal data associated (Peripheral serum, EDTA-buffy coat, EDTA-plasma, Li Hep-plasma, urine, stool, saliva and gastro intestinal biopsies). For this reason, international, European and local policies on human data samples and associated personal data are applicable.

The principle investigator should explain to potential participants the purpose of the study and the scientific and possible therapeutic value of the uses of their human biological samples for research purposes. This information could be useful for having more science-based knowledge on the progression of the disease, related to cirrhosis. Altruism and solidarity are the principles that form the basis of our health care system, based on sound research and promoting translational medicine, personalized treatments and interventions. Potential participants in research should consent to donate, to store and use their biological samples in the entitled biobanks: as part of the biobank or collection regime, they should be informed about possible access of third parties and the time for how long the samples will be stored. Full contact of biobanks (including contact person in charge and address) should be provided. The principles of quality, security, safety and traceability should be respected, and proper confidentiality measures to protect involved human beings should be taken. The participants should be informed about the use of leftover materials, and that their samples are going to be codified, pseudonymized or completely anonymized depending on the purpose of the research. In the case of codified samples, participants should be informed that the samples will be frozen and stored with a number assigned to them instead of their names. Since the samples are codified, the owners of the material can revoke consent for the use of them at any time, but they should be aware as well that the information, the data generated for scientific purposes, will remain.

The information process and informed consent procedures for conducting research with human biological samples should include the information that the research itself could result in new products, tests or discoveries which may have commercial value. Donors should be informed and clearly understand that they do not retain any property rights to the materials nor any financial benefits from these products, tests or discoveries.

In case of incidental findings or any new information discovered during the research process that could affect the entitled person this should be properly managed: From the beginning, participants should be informed about the possibility of being contacted if there is relevant health-related information that affects them and/or their relatives. Main researchers should follow national and local regulations on the issue. Participants should be informed that any research based on their samples is reviewed by the entitled research ethics committees, and that biobanks that store these human biological samples have scientific and ethical committees evaluating and monitoring the whole process, including data transfers if any, to assure the protection of their rights, specifically privacy and confidentiality.

6. Information about publication of MICROB-PREDICT results

The study participants should be informed that data and research results of the MICROB-PREDICT project may be published. No personal information revealing the identity of participants will be published. Privacy and confidentiality will always be assured and research integrity is a priority. All Consortium members are obliged to follow the European Code of Conduct for Research Integrity, Allea, 2017 in order to assure research integrity and avoid research misconduct and other questionable practices from the inception and during the whole process of research.

7. Information about Doctoral Thesis in the framework of MICROB-PREDICT

In the case of doctoral theses to be developed in the frame of the MICROB-PREDICT project, participants should be informed about this situation and PhD candidates and supervisors should be clearly identified. Both are bound to follow the above described requirements, and participants have the right to know what kind of activities and interventions in the field of research are going to be developed by the PhD candidate. Doctoral thesis projects should as well be reviewed by the entitled Research Ethics Committee.

8. Main international and European ethical and legal guidelines and regulations for conducting research in human beings

From the legal perspective:

- The Universal Declaration on Bioethics and Human Rights, UNESCO, 2005. http://www.unesco.org/new/en/social-and-human-sciences/themes/bioethics/bioethics-and-human-rights/
- The Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine (ETS No 164) was opened for signature on 4 April 1997 in Oviedo (Spain).

(The Oviedo Convention on Biomedicine and Human Rights, Council of Europe, 1997) https://www.coe.int/en/web/bioethics/oviedo-convention

- The European Charter of Fundamental Rights, European Union, 2000 https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:12012P/TXT
- Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32014R0536
- Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation-GDPR) https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32016R0679

From the ethical perspective:

- The Helsinki Declaration, World Medical Association, 2013 https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/
- European Code of Conduct for Research Integrity, Allea, 2017 https://www.allea.org/allea-publishes-revised-edition-european-code-conduct-research-integrity/
- Ethics and Data Protection. See the lastest guide on the issue published in november 2018 after the application of the General Data Protection Regulation http://ec.europa.eu/research/participants/data/ref/h2020/grants_manual/hi/ethics/h2020_hi_ethics-data-protection_en.pdf

Moreover, MICROB-PREDICT is respecting all national and local guidelines and regulations applicable to all research settings.

Appendix V – Model informed consent forms