CLINICAL TRIAL PROTOCOL

HALT

Influence of Human Albumin supplementation on kidney dysfunction after Liver Transplantation

Category of research: Clinical Trial on medicinal products for human use	
Sponsor protocol number: 35RC22_9739_HALT	EU CT n° : 2024-514804-14-00
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VERSION 1.1 dated 12.12.2024

SIGNATURE PAGE

Coordinating investigator

I have read all pages of this clinical trial protocol for which the University Hospital of Rennes is the sponsor. I confirm that it contains all the information necessary for the correct conduct of the trial. I agree to conduct the trial, in compliance with the protocol and the terms and conditions defined therein. I agree to conduct the trial in compliance with the applicable laws, regulations and good clinical practice.

- The principles of the "Declaration of Helsinki",
- The rules and recommendations of international (ICH-E6) and French good clinical practice (règles de bonnes pratiques cliniques pour les études de recherche biomédicale portant sur les médicaments à usage humain - décisions du 24 novembre 2006),
- national legislation and regulations relating to clinical trials,
- The Clinical Trials Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 relating to clinical trials on medicinal products for human use and repealing Directive 2001/20/EC.

I also agree that the investigators and other qualified members of my team may have access to copies of this protocol and documents pertaining to the correct conduct of the trial, enabling them to work in compliance with conditions contained in these documents.

NOM : Pauline HOUSSEL DEBRY Date : le 05/02/2025

Signature :

SPONSOR

NOM : Julie COURPRON – Clinical Research Department Director – CHU of Rennes

Signature : Date : le 6/02/25

VERSION HISTORY

The version history concerns only authorized and therefore current versions (ANSM/CPP).

The 1st version submitted to the authorities (ANSM/CPP) will be version 1.0 dated DD/MM/YYYY.

In the meantime, in order to keep track of working versions, the protocol is versioned in 0.x before submission.

Version	Date	Subject
1.0	04.10.2024	Initial authorization
1.1	12.12.2024	Response to RFI / Authorised version

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LIST OF ABBREVIATIONS

AKI: Acute Kidney Injury

AKIN: Acute Kidney Injury Network

ALT: Alanine aminotransferase

ANSM: Agence nationale de sécurité du médicament et des produits de santé

aOR : Adjusted Odd Ratio

AST: Aspartate aminotransferase

CHU: Centre Hospitalier Universitaire

CI: Confidence interval

CNIL: Commission nationale de l'informatique et des libertés

CPP: Comité de Protection des Personnes

CRA: Clinical Research Associate

DRI : Direction de la Recherche et de l'Innovation

EASL : European Association of the Study of the Liver

eCRF: electronic Case Report Form

eGRF : Estimated Glomerular Filtration Rate

EU : European Union

EU CT : European Union Clinical Trial

GCP: Good Clinical Practice

GGT: Gamma-Glutamyl Transferase

ICH: International Conference on Harmonisation

ICU: Intensive Care Unit

IEC: Independent Ethics Committee

INR: International Normalized Ratio

INSERM: Institut national de la santé et de la recherche médicale

KDIGO: Kidney Disease: Improving Global Outcomes

LPLV: Last Patient, Last Visit

LT: Liver Transplantation

MDRD: Modification of Diet in Renal Disease

MELD: Model for End-Stage Liver Disease

MR: Methodology of Reference

NCT : National Clinical Trial

OLT: Orthotopic Liver Transplantation

OR : Odd Ratio

RIFLE: Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease

SAE: Serious Adverse Event

SAS: Statistical Analysis System

SOP: Standard Operating Procedure

TAC: Tacrolimus

SUMMARY

1 BACKGROUND AND RATIONALE

1.1 CONTEXT

Human Albumin has many important physiologic properties and has been used in numerous clinical purposes. Preclinical studies in cirrhotic patients suggest that perfusion of albumin might limit systemic inflammation, prevent infections, reduce the risk of kidney dysfunction, and increase survival. Although patients undergoing liver transplantation (LT) are hypoalbuminemic, there is no data supporting albumin administration after LT and these practices are not evidence-based (level recommended: 25g/l). However, there are several reasons which can easily support albumin administration, especially the improvement of kidney function after LT, the maintenance of normal plasma albumin concentrations to return drug binding to normal and to diminish occurrence of calcineurin inhibitor associated neurological side effects. (1)

1.1.1 POSTOPERATIVE COMPLICATIONS AFTER LIVER TRANSPLANTATION

Patients who undergo orthotopic liver transplantation spend some time in the intensive care unit (ICU) during the postoperative period with an expectation of 24 hours of postoperative mechanical ventilation and an average stay of 6 days in ICU for routine cases before discharge to surgery ward. Because of preexisting conditions such as recipient age and Model for End-Stage Liver Disease (MELD), intraoperative events that may induce postoperative complications, the stay is more prolonged (2; 3; 4)

Among postoperative complications, infectious complications are the most common causes of early post-transplant morbidity and mortality and are associated with ICU length of stay (5). Acute kidney injury (AKI) are common after LT. The occurrence of AKI in patients undergoing LT is associated with reduced patient and graft survival not only in the perioperative period but also in the longer term (4; 5; 6). The etiology of AKI after LT is multifactorial, including baseline renal dysfunction, acute or chronic respiratory failure, absolute hypovolemia, perioperative circulatory failure, massive transfusion, use of immunosuppressive or nephrotoxic drugs, graft dysfunction, and sepsis (postoperative infections). In a retrospective analysis, Sang et al found that **early postoperative hypoalbuminemia was an independent risk factor for acute kidney injury**. Furthermore, post transplant AKI is associated with longer stays in the intensive care unit, increased graft rejection, higher hospital costs, and higher mortality independent of pretransplant renal function (7; 8; 9).

1.1.2 ALBUMIN ADMINISTRATION AFTER LT

Albumin is a major multifunctional protein. It accounts for 55-60% of all plasma proteins. The physiological functions of albumin include maintenance of colloid osmotic pressure, binding and transporting a large number of endogenous and exogenous substances, acid-base function, anti-oxidant and free radical scavenging, anti-apoptotic effects, effects on vascular integrity, anti-coagulant and anti-thrombotic properties (8; 9). Albumin is synthetized by hepatocytes at a rate between 12 and 25 g per day. Albumin is known to be a marker of nutrition, inflammation, hepatic function, and overall catabolic state**. Hypoalbuminemia is generally defined as a serum albumin concentration < 30 g/L** (10; 11)**.** Whatever the underlying mechanism, hypoalbuminemia is associated with worst outcome in critically ill patients and there is a clear relationship between the albumin level and the severity of the insult (9; 11; 12). However, correction of low serum albumin levels in these patients did not improve outcome (12; 13). For instance, in patients with severe sepsis, albumin replacement in addition to crystalloids, as compared with crystalloids alone, did not improve the rate of survival at 28 and 90 days (14). In this study, patients were randomly assigned to receive either 20% albumin and crystalloid solution (albumin group) or crystalloid solution alone (crystalloid group) from randomization until day 28 or discharge from the ICU. After randomization, patients in the albumin group received 300 ml of 20% albumin solution. From day 1 until day 28 or ICU discharge (whichever came first), 20% albumin was administered on a daily basis, to maintain a serum albumin level of 30 g per liter or more. Noteworthy, the serum albumin level was significantly higher in the albumin group than in the crystalloid group from day 1 to day 28 and the study goal was achieve within 24 hours (P<0.001) (Figure 1). (14)

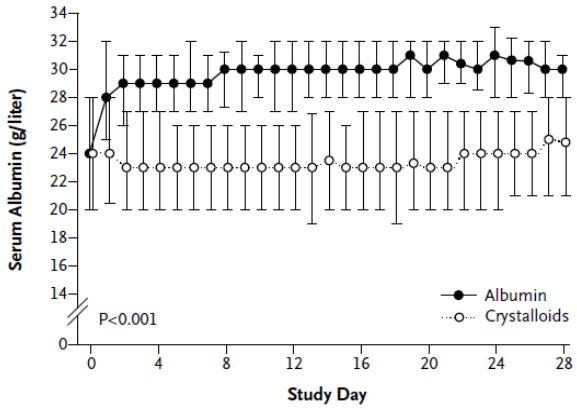


Figure 1: Serum albumin levels through day 28 can be restored within 24 hours following albumin administration (14)

On the contrary, in patients with chronic liver disease (cirrhosis), correction of hypoalbuminemia leads to a decrease in both morbidity and mortality in particular situations (8; 12; 15; 16). Several studies have demonstrated that albumin administration has to be considered in the following conditions:

- Patients with cirrhosis and spontaneous bacterial peritonitis
- Patients with cirrhosis and type 1 hepatorenal syndrome
- Patients with cirrhosis and large volume paracentesis

Patients with end-stage liver disease referred for LT surgery are often hypoalbuminemic, due to malnutrition, having decreased hepatic synthesis, or altered distribution of albumin between

intravascular and interstitial compartment by increased vascular permeability during major surgical procedures (2; 10; 17; 18). Albumin serum after OLT remains low for several days as reported in numerous studies. In an observational study, Sang et al found that postoperative albumin remained below 30 g/L and in a prospective study, Mukhtar et al found that patients after OLT showed an albumin serum level of 25 g/L after surgery which remained very low in the following weeks (6; 19).

Although there is no data supporting it, actual practices recommend administration of exogenous albumin during and after LT for volume substitution and with the intention of maintaining plasma albumin level above 25 g/L (2; 17; 20)**.** A rational approach aiming at maintaining circulating volume is by providing required fluids with crystalloid and albumin. The real advantage of albumin solutions on the final outcome is still under debate after LT while the use of human albumin solution, coupled with low-sodium fluids has the potential to restore oncotic pressure, promote intravascular mobilization of fluid and prevent an excessive increase in plasmatic sodium (9). Furthermore, maintaining higher levels of serum albumin could facilitate immunosuppressive treatment, decrease their toxicity and decrease postoperative kidney injury after LT (10; 15; 21). Lastly, albumin administration can restore albumin serum albumin level within 24 hours following LT. For instance, in a prospective study aiming to maintain serum albumin level >30g/l, patients received 20% albumin (average volume 300ml/day) with a clear impact on serum albumin levels (19).

1.1.3 POSTOPERATIVE HYPOALBUMINEMIA AND KIDNEY INJURY

Acute kidney injury (AKI) is a major complication of LT, and its prevalence has shown a wide range of between 17% and 95% because of different definitions of AKI (10). Furthermore, postoperative AKI has been related to postoperative ICU stay and mortality after LT (7; 22). Previous reports have shown that hypoalbuminemia is associated with an increased risk of AKI and is an independent risk factor for morbidity and mortality in critically ill patients and in various surgical situations (6; 12; 23). Several studies have demonstrated that preoperative serum albumin level was associated with acute kidney failure. For instance, Cabezuelo et al previously reviewed 184 consecutive patients who underwent LT and found a correlation between a low preoperative serum albumin level (< 3.2 g/dL) and AKI after LT (7). In addition, Chen et al reported that preoperative hypoalbuminemia (< 3.5 g/dL) was a strong predictor of postoperative AKI in patients undergoing LT (11; 24). Furthermore, a recent study has demonstrated that postoperative minimal albumin level was an independent risk factor for acute kidney injury defined by both the AKIN and the RIFLE criteria, and was related to overall mortality after liver transplantation (6; 25; 26). However, no prospective study has evaluated the effect of albumin in prevention of postoperative kidney injury after liver transplantation.

Although a detailed mechanism of action by which albumin has a positive influence on renal function has not yet been completely elucidated, studies have suggested that albumin might have

renoprotective effects through antioxidant properties, ability to scavenge reactive oxygen species and decrease apoptotic rate in renal tubular cells (6; 8; 18).

1.1.4 IMMUNOMONITORING

As albumin has been shown to counteract immune-suppressive effects induce by advanced Liver Disease, immune markers associated with morbidity and mortality in patients admitted in ICU will be studied. Along these lines, immunological parameters will study tacrolimus-associated effects on lymphocytes at day 1, day 3 and day 7. Immune parameters will be compared to baseline (before Orthotopic Liver Transplantation, OLT, at the beginning of surgery). Although therapeutic drug monitoring (TDM) of immunosuppressive drugs is used to improve the immunosuppressive effect while minimizing the toxicity related to exposition to high serum levels, **immunological effects depend on unbound tacrolimus (TAC).** Investigation of the mechanisms of action of TAC has provided biomarkers useful for monitoring the immunological effects. Flow-cytometry-based measurement of lymphocyte proliferation, T-cell surface markers, and cytokines has been performed in stimulated whole-blood cultures derived from humans.

In this study we will monitor the immune effects of immunosuppression on lymphocyte after OLT. Among parameters studied, we will analyze lymphocyte proliferation, cytokine production (interleukin-2 (IL-2), IL-10, IL-6 and tumor necrosis factor-alpha (TNF-α)), and IL-2 mRNA expression. Furthermore, cytometry will be performed on fresh whole blood to evaluate changes in cell populations (neutrophils, MDSC, T-cells, B-cells and monocytes) before liver transplantation (D0), D1, D3 and D7 after OLT. The pharmacological effects of tacrolimus in calcineurin dependent and calcineurin independent (mitogen-activated protein kinase (MAPK) dependent) activation pathways will be assessed by measuring activated nuclear factor of activated T cells (NFAT) and p38, respectively, by flow cytometry. To also study **the effect of plasma from patients after OLT,** we will expose lymphocytes from healthy donors to plasma from patient after OLT to study induced changes.

1.2 NAME AND BRIEF DESCRIPTION OF THE CONDITION AND OF THE TARGET POPULATION

Recipients of primary liver allografts from a deceased donor and as a single organ (liver only) will be studied. These subjects will have a liver disease with indication of LT. These subjects have a persistently low serum albumin concentration during the early postoperative period when recovery of synthetic and metabolic functions of the grafted liver can be slow.

1.3 NAME AND BRIEF DESCRIPTION OF THE MEDICINAL PRODUCT / STRATEGY UNDER INVESTIGATION

Treatment with human Albumin for cirrhosis complications is one of the well evaluated indications in clinical trials and remains among the most recognized indications for the use of Albumin

The use of Albumin in the drainage of ascites, particularly in large volume (>4L), was evaluated in a meta-analysis including 857 subjects from 17 studies. In this analysis, Albumin had a highly favorable effect on the occurrence of post paracentesis hypovolemia (OR: 0.39 [0.27-0.55]), hyponatremia (OR: 0.58 [0.39-0.87]) and mortality (OR: 0.64 [0.41-0.98]). These favorable results have led the European Association of the Study of the Liver (EASL) to recommend Albumin in this indication (8g/L of ascites) (27; 28).

Albumin is recommended for the treatment of spontaneous bacterial peritonitis at a dose of 1.5 g/kg at the time of diagnosis and 1 g/kg at D3 (4 clinical trials). In this indication, it has shown a positive effect on the occurrence of kidney failure and on mortality. Albumin is also recommended in cases of hepatorenal syndrome in the context of volemic expansion and in association with the use of vasoconstrictor agents.

1.4 RESULTS OF PREVIOUS STUDIES

Although low albumin levels have been associated with mortality in different clinical settings (8; 11), a recent Cochrane review concluded that albumin administration should be used cautiously (12): “For patients with hypovolaemia, there is no evidence that albumin reduces mortality when compared with cheaper alternatives such as saline. There is no evidence that albumin reduces mortality in critically ill patients with burns and hypoalbuminaemia. The possibility that there may be highly selected populations of critically ill patients in which albumin may be indicated remains open to question. However, in view of the absence of evidence of a mortality benefit from albumin and the increased cost of albumin compared to alternatives such as saline, it would seem reasonable that albumin should only be used within the context of well concealed and adequately powered randomised controlled trials.”

On the contrary, albumin administration in patients with chronic liver disease (cirrhosis), correction of hypoalbuminemia leads to a decrease in both morbidity and mortality in particular situations. (8; 12; 15).

- Serum albumin levels are low after OLT and could be corrected within 24h using Human Albumin 20% solution (19).
- Low serum albumin levels may be responsible for tacrolimus-associated neurotoxicity due to increase unbound fraction (19; 29; 30; 31; 32).

- Low serum albumin levels have been associated with occurrence of acute kidney injury after LT in several studies although no prospective study confirms these findings (18).
- We conducted a retrospective analysis of 230 LT within the last 2 years to study the association between low albumin levels after LT and TAC associated neurological events: (cf Appendix). Recipients of primary liver allografts from a deceased donor and as a single organ (liver only) with the use of TAC as immunosuppressive therapy were included.

Neurological events were defined as follow: encephalopathy appearing or persisting after 7 days following OLT, confusion, tremor, seizures and posterior reversible encephalopathy syndrome.

We found an association between postoperative neurological complications and hypoalbuminemia (within 7 days following LT)

We also studied the occurrence of other postoperative complications such as acute kidney injury (AKI). AKI was diagnosed using AKIN classification (alteration of the serum creatinine (sCr) concentration on postoperative 1 to 7 days when compared with that of the baseline sCr concentration, defined as the last concentration measured prior to surgery). We found an association between hypoalbuminemia and occurrence of AKI. Of note, we found that hypoalbuminemia was associated with the need for renal replacement therapy.

1.5 BENEFIT-RISK BALANCE

1.5.1 BENEFITS

1.5.1.1 Individual benefits

Expected patient benefits are:

- A significant decrease in the occurrence of AKI
- A significant decrease in the ICU length of stay
- A significant decrease in hospital length of stay
- A significant decrease in both mortality and morbidity
- A significant decrease in the occurrence of tacrolimus associated neurological side effects
- A significant decrease in the occurrence of postoperative infection due to shorter ICU and hospital length of stay
- A significant decrease in exposure to antibacterial agent, which could be harmful (as clostridium difficile colitis)

1.5.1.2 Collective benefit

Expected public health benefits are:

- Significant decrease in hospital stay will significantly reduce cost associated to liver transplantation
- Clear benefits for public health since healthcare associated infections in hospitals impose significant economic consequences on the nation's healthcare system. A decrease in the emergence of resistant strains in surgery, hospital and general population since postoperative infections are responsible for antimicrobials prescription, which is the single most important cause of the emergence of drug resistance, both in the community and hospital settings.
- To obtain novel mechanistic insights for understanding the immune landscape in liver transplantation

1.5.2 RISKS

No specific risks (individual or collective) are likely to occur since several studies have found benefits to maintain albumin serum above 30g/L, mainly in biological parameters.

1.5.2.1 Physical risks

Main constraints are blood withdrawn from subjects. However, volume withdrawn will not induce any physical side effect.

Albumin contributes more than 80% to plasma oncotic pressure. Therefore, the use of colloids and in particular of albumin in excess could lead to acute pulmonary oedema.

1.5.2.2 Psychological and socio-economic risks

The subjects will not present any specific psychological risks or constraints associated with this study. The psychological risks to the subjects included in this trial are the same as for any subject requiring liver transplantation.

The subjects will not present any specific socio-economic risks or constraints associated with this study.

1.5.3 BENEFIT-RISK RATIO

Acute kidney injury is a common and critical complication of liver transplantation, which is associated with increased morbidity, mortality and health care cost. Previous studies have reported that AKI after LT is not only associated with immediate complications including volume overload, metabolic acidosis, and electrolyte disturbances, but also an increased rate of inferior long-term outcomes such as mortality, graft loss, infection, chronic kidney disease, prolonged stay in the intensive care unit, and increased hospital costs.

Previously, Berkowitz and al demonstrated that low serum albumin <30 g/l (aOR, 0.576; 95% CI, 0.410–0.808; p = 0.001) was associated with AKI after LT. (33)

The perfusion of human Albumin after LT will correct hypoalbuminemia. This volemic expansion may control AKI and improve the post-transplant outcomes.

Given the expected benefits and low specific risks, the benefit/risk ratio is highly favourable.

2 OBJECTIVES AND OUTCOME MEASURES

2.1 OBJECTIVES AND OUTCOME MEASURES

2.1.1 PRIMARY OBJECTIVE

To verify whether albumin administration to achieve serum concentration above 30g/L (treated group) and its maintenance within plasmatic physiologic range (above 30 g/L) for five days diminishes rate of AKI at Day 7 after liver transplantation as compared to restrained albumin administration (when serum concentration is at 20 g/L or below (control)).

2.1.2 PRIMARY OUTCOME MEASURE

Occurrence of AKI during the first 7 days after liver transplantation (Cf Appendix for AKI definition according to KDIGO criteria)

2.2 SECONDARY OBJECTIVES AND OUTCOME MEASURE

To verify whether albumin administration to achieve serum concentration above 30 g/L (treated group) and its maintenance within plasmatic physiologic range (above 30 g/L) for five days diminishes as compared to restrained albumin administration (when serum concentration is at 20 g/L or below (control)).

- Occurrence and severity stages of AKI during the first 7 days after liver transplantation
  - ➔ Occurrence and Severity of each AKI during the first 7 days after liver transplantation (Cf Appendix for AKI definition and stages of severity according to KDIGO criteria) (34)

- hospital length of stay
  - ➔ Hospital length of stay after liver transplantation *defined by standardized checklist of discharged criteria* (35)

- occurrence of calcineurin inhibitor induced neurotoxicity,
  - ➔ Postoperative Tacrolimus-induced neurotoxicity at D28
    which will include: encephalopathy appearing or persisting after OLT, confusion, tremor, seizures and posterior reversible encephalopathy syndrome with severity classification according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0,

- occurence of calcineurin inhibitor withdraw,
  - ➔ Discontinuation of anticalcineurin inhibitor at D28.

- occurrence of postoperative infections,
  - ➔ occurrence of postoperative infections at D28,

    The diagnosis of a postoperative infection will be based on clinical, biochemical, or morphological features and confirmed (if possible) by bacteriological data according to CDC definitions for nosocomial infections

- occurrence of acute graft rejection
  - ➔ Acute graft rejection (biopsy proved) at D28

- occurrence of early graft dysfunction,
  - ➔ occurrence of early graft dysfunction,

    EAD was defined as the presence of one or more of the following previously defined postoperative laboratory analyses reflective of liver injury and function:
    - *bilirubin >or=10mg/dL on day 7,*
    - *international normalized ratio >or=1.6 on day 7,*
    - *and alanine or aspartate aminotransferases >2000 IU/L within the first 7 days*

      (critères d'Olthoff Liver Transplantation 2010) (36)

- duration of mechanical ventilation,
  - ➔ Duration of mechanical ventilation at D28

- reintubation rate,
  - ➔ occurrence of reintubation at D28

- ICU length of stay
  - ➔ Duration of ICU length of stay up to D28

- and ICU readmission rate,
  - ➔ occurence of ICU readmission after discharge of ICU unit up to D28

- All cause mortality

➔ Mortality within the first 28 days following surgery

Secondary objectives related to Immunomonitoring (Rennes subjects only):

To study whether albumin administration will:

- decrease the occurrence of lymphopenia after surgery
  - ➔ Defined as a lymphocyte count less than 1.2× 10^3 cells/µL
- increase lymphocyte proliferation rate and decrease postoperative lymphocyte apoptosis
  - ➔ Using flow cytometry
- improve lymphocyte mitochondrial function
  - ➔ Using the Seahorse analyzer measuring mitochondrial respiration
- decrease postoperative immunosuppression
  - ➔ Measured using expression of HLA-DR on peripheral monocytes, plasmatic levels of IL-10, MDSC, RNA seq analysis on peripheral blood mononuclear cell
- decrease postoperative inflammation
  - ➔ Plasmatic levels of IL-6

3 TRIAL DESIGN

3.1 EXPERIMENTAL DESIGN

A prospective, multicenter, randomized, controlled, open blinded, superiority trial performed on two parallel groups.
Informed consent will be obtained before liver transplant (at listing or during the follow up before LT).

After the first biological test following LT, subjects will be randomly (ratio 1:1) assigned to receive either Human Albumin 20% Solution :

- when albumin serum concentration is at 30 g/L or below (treated group)
- when albumin serum concentration is at 20 g/L or below (control group)

The randomisation is performed regardless of the initial albumin concentration of the patient.

Albumin serum concentration will be measured once a day (on mornings), at fixed time everyday (centers will be able to decide the timing of blood samples according their current practices). As soon as the results are received, human Albumin 20% Solution will be perfused. A calculator will be available to determine the exact amount of Albumin to perfuse. (cf § dosage adjustment)

After day 5, albumin will be infused according to center's current practice.
Subjects will be followed until day 28.

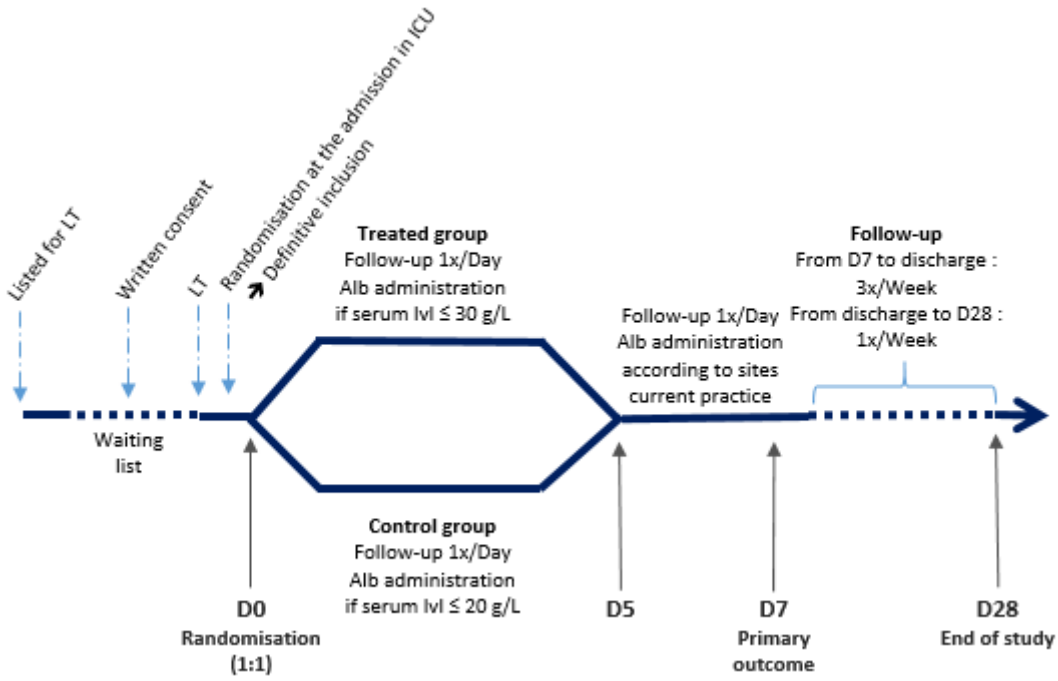
3.2 DURATION OF THE RESEARCH

Recruitment period:	36 months
Duration of subject monitoring:	28 days
Duration of analysis of data:	6 months
Estimated total duration of study:	43 months

3.3 DEFINITION OF THE BEGINNING AND END OF THE RESEARCH

The first act of recruitment in this trial is the signing of consent by the first subject in the trial.
The end of the research is defined as the last visit by the last participant (LPLV).

3.4 STUDY DESIGN



4 RECRUITMENT, ELIGIBILITY AND CONSENT

4.1 RECRUITEMENT

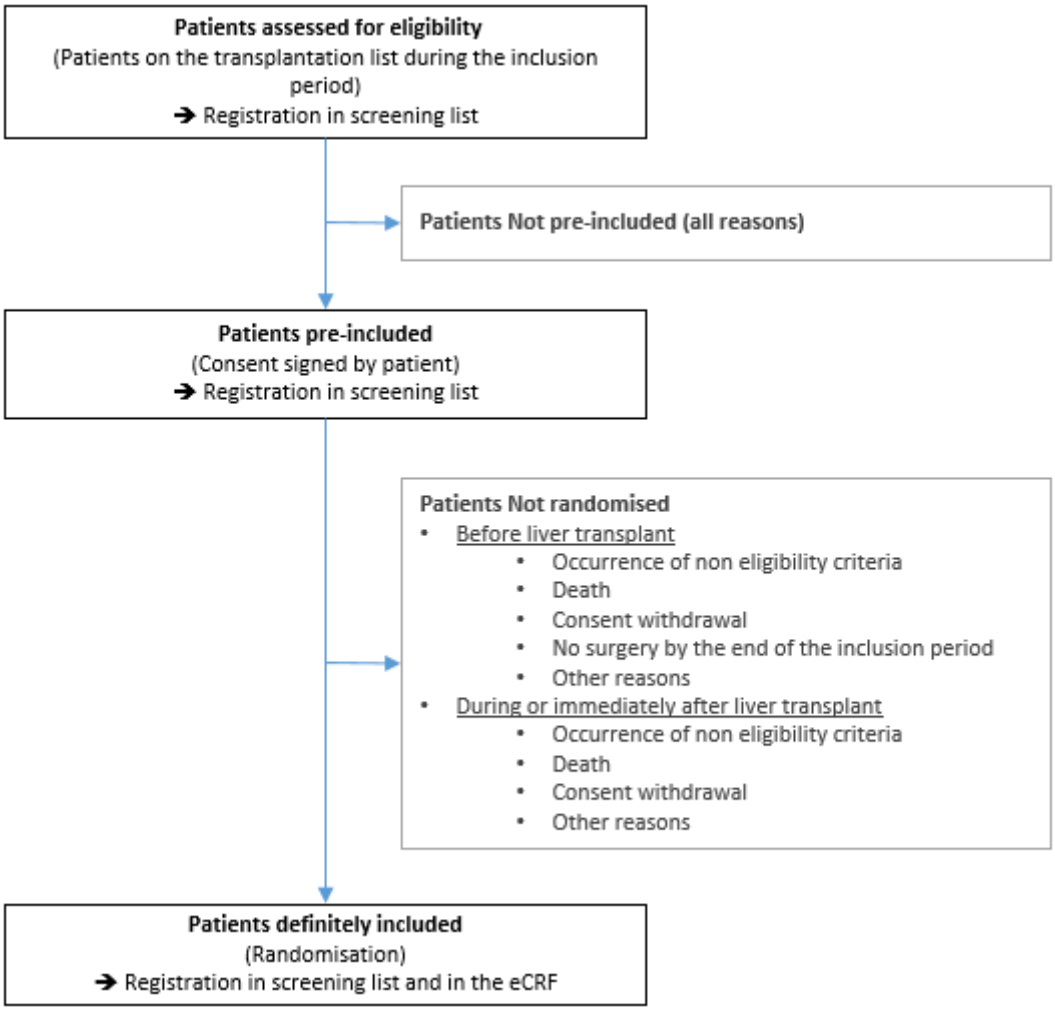
Patients listing for LT may be offered participation in the study. Eligible patients according to the inclusion criteria will be recruited during standard visits. Informed consent along with a document describing the study will be presented during visit. The informed consent will be mandatory obtained to include the patient in this research protocol.

Patients whom have signed the informed consent will be considered as pre-included.

Patients will be considered definitely included once the randomisation is performed.

4.2 SCREENING/SELECTION

The enrolment will be performed following the following process. The number of subjects in each category will be notified in compliance with the CONSORT flow-chart.


Patients assessed for eligibility
(Patients on the transplantation list during the inclusion period)
➔ Registration in screening list
Patients Not pre-included (all reasons)
Patients pre-included
(Consent signed by patient)
➔ Registration in screening list
Patients Not randomised
Before liver transplant
Occurrence of non eligibility criteria
Death
Consent withdrawal
No surgery by the end of the inclusion period
Other reasons
During or immediately after liver transplant
Occurrence of non eligibility criteria
Death
Consent withdrawal
Other reasons
Patients definitely included
(Randomisation)
➔ Registration in screening list and in the eCRF

4.3 INCLUSION CRITERIA

- IC1 - Male and female subjects equal or above 18 yrs old.
- IC2 - Recipients of primary liver allografts from a deceased donor (including after cardiac death) and as a single organ (liver only).
- IC3 - Capability of understanding the purpose and risks of the study.
- IC4 - Written informed consent

4.4 NON INCLUSION CRITERIA

- NIC1 - Fulminant hepatitis
- NIC2 - Kidney injury at baseline (eGFR < 50 ml/min/1.73m² in MDRD-6) including hepatorenal syndrome
- NIC3 - Use of an induction agent Basiliximab at liver transplantation
- NIC4 - Protected person (adults legally protected, under judicial protection, guardianship, or supervision), person deprived of their liberty
- NIC5 - At the time of randomisation, participation to another interventional study

4.5 INFORMATION AND CONSENT

Potential participants will receive oral information about the study and a written information letter.
Participants will be fully and fairly informed, in understandable terms, of the objectives and constraints of the study, the possible risks involved, the necessary monitoring and safety measures, their rights to refuse to participate in the study or of the possibility of retracting their agreement at any time.
In case the potential participant does not speak French, a translator may be called in to help.
The participant's free, informed and written consent will be obtained by the investigator, or a physician representing the participant, before liver transplant
The information letter and consent form will be signed by the participant and the investigator. The participant will retain the information letter and the copy of the consent, the investigator will retain the original.

Once the consent form signed by the patient, he will be considered as pre-included

4.5.1 SPECIFIC PROCEDURES FOR OBTAINING CONSENT

If the patient is unable to write due to a physical disability and wishes to participate in a free and informed manner and has expressed this explicitly, a third person present may represent the patient and sign the consent in the specific box provided. The subject's oral consent, the name and signature of the third party and the reasons for this procedure are documented in the subject's medical record.

5 CONDUCT OF THE STUDY

5.1 LIVER TRANSPLANT

A plasmatic albumin assessment will be performed before the liver transplantation. The liver transplantation will be performed according to the site practice.

5.2 ENTRANCE IN INTENSIVE CARE UNIT AND RANDOMISATION

At the entrance in the ICU, eligibility criteria will be checked and the subject will be randomised in one of the arms of the study.
The randomisation will be performed by the investigator, via a webinterface (Ennov Clinical, Ennov Group, Paris, France). A document to help using this tool will be provided to the investigators. (For more details one randomisation, see §9.1).

Once randomised, the subject is considered as definitely included.

The first albumin perfusion will be performed in accordance with the strategy defined by the randomisation.

5.3 FOLLOW-UP (FROM D1 TO D28)

5.3.1 IMMUNOSUPPRESSIVE REGIMEN

For both the standard and modified therapy groups, tacrolimus will be administered within 12 h post-transplant at an initial dose of 0.03 mg/kg bid, with a subsequent dose adjustment to achieve whole blood trough levels between 5 and 10 ng/mL. In addition, both groups will receive prednisolone and mycophenolate mofetil according to the current practice of the site.

5.3.2 CLINICAL EXAM / PARACLINICAL INVESTIGATIONS AND OTHER DATA REPORTED IN THE ECRF

Clinical exam / Paraclinical investigations and other data reported in the eCRF	
MELD Score	➔ Before transplantation
Initials / Birth date (Month/Year) / Gender	➔ Randomisation
Medical History	➔ Inclusion
Child-Pugh score	
Data related to anaesthesia	
Data related to surgery	
Data related to the donor and the graft	
Albumin administration	➔ During LT and up to D7
Acute Renal Failure	➔ Until D7
Early graft dysfunction	➔ Until D7
Acute graft rejection	➔ In case of occurrence until D28
Postoperative infections	
Tacrolimus associated neurological side effects	
ICU length of stay	➔ until D28
ICU readmission	
Duration of mechanical ventilation	
Reintubation rate	
Hospital length of stay	
Mortality	
Treatments	➔ From randomization to D28
Adverse Events	

5.3.3 BIOLOGICAL ASSESSMENTS

Biological assesments	
Arterial blood pressure	- First 7 days : Daily
Pulse rate	
Respiratory rate	
Urine output	
Fraction of inspired oxygen (F_IO_2)	➔ Daily, as long as the subject is under mechanical ventilation
Albumin (during the morning)	➔ On leaving the operating room ➔ First analysis of the day ➔ First 7 days : Daily ➔ After D7, according to the site practices and up to D28
Hematocrit / Hemoglobin.	➔ First analysis of the day ➔ First 7 days : Daily ➔ During Hospital Stay : 3x / week ➔ After discharge : 1x / week
Bilirubin (Total and Direct)	
AST, ALT	
Total protein	
Tacrolimus whole blood trough concentration	
Serum levels of creatinine	
Creatinine clearance (CKD-EPI creatinine equation)	
Urea	
Taux de prothrombine (TP)	
INR	
Immunonitoring	➔ Before LT ➔ Maximum 24h after LT ➔ At D3 ➔ At D7

5.3.3.1 *Procedures for collection*

All samples will be collected through venous sampling

Routine care (may vary depending on each site practices)

- 1 Heparin Lithium tube (3 mL of blood collected) : Bilirubin Total and Direct, AST, ALT, Total protein, Creatinine, Urea
- 2 EDTA tubes (2x2 mL of blood collected) : Hematocrit / Hemoglobin, Tacrolimus
- 1 Citrate tube (2.7 mL of blood collected) : TP

Research procedures :

- Albumin analysis will be performed using the same Heparin tubes collected in the routine care.
- Rennes subjects only : samples collected for immunomonitoring study are described in §5.4

5.3.3.2 Volume of blood collected

	Per Procedure	Entire Study (28 days)
Routine Care (may vary depending on each site practices)	9.7 mL	155.2 mL maximum (16 procedures maximum)
Albumin (Research Purpose)	Not applicable	Not applicable
Immunomonitoring (Rennes only) (Research Purpose)	26.5 mL	106 mL

5.3.3.3 Biopsies

In case of suspicion of acute graft rejection, sites will perform liver biopsies according to their own practices.

5.4 IMMUNOMONITORING STUDY (RENNES SUBJECTS ONLY)

In order perform an immunomonitoring analysis, whole blood a sample of 26.5mL (6 x 4 mL heparin tubes and 1 x 2.5 mL Paxgene tube) will be taken:

- Before transplantation
- Within 24 hours of transplantation
- at D3
- at D7

Therefore a total of 106mL of whole blood will be sampled during the study for each patient.

Once collected, the samples will be sent to the SITI laboratory where :

- The Paxgene tube will be stored at -80°C
- 1 Heparin tube will be used for cytometry
- 5 Heparin tubes will be separed and used for :
    - plasma collection, stored in maximum 6 labelled cryotubes (500 µL) at -80°C.
    - and PBMC collection, stored in maximum 6 labelled cryotubes (1mL) at -196°C.

The following analysis will be performed:

5.4.1 CYTOMETRY

Cytometry will be performed on fresh whole blood to evaluate changes in cell populations. Several antibody cocktails will be used to study the different cell populations: **neutrophils** (CD66b, CD35, CD11b, CD63, CD88, CD16, CD64, CD15, CD45), **GMDSC** (CD3, CD15, CD45, CD11b, CD14, CD19, CD20, CD56, CD123, CD33, HLA-DR), **regulatory T-cells** (CD127, HLA-DR, CD39, CD45-RA, CD25, CCR7, CD3, CD4, CD8, Foxp3, Ki-67), **MMDSC** and **monocytes subpopulations** (CD3, CD16, CD66b, CD56, CD335,

CD45, CD274, CD14, HLA-DR, CD169), **B cells** (Lag3, CD138, CD39, CD69, CD45, CD20, CD1d, CD27, CD38, CD25, CD24, CD3, CD19, CD5), **T-cells** (HLA-DR, CD56, CD16, CD45-RA, Tim-3, Lag3, PD-1, CCR7, CD3, CD4, CD8).

T-cell apoptosis will be evaluated in cytometry by using active caspase-3 labelling.

5.4.2 METABOLOMIC PROFILING

Metabolomic profiling of plasma will be measured by LC/MS technology. Approximately 150 metabolites will be quantified allowing to study several pathways as glycolysis, TCA and urea cycle, fatty acid metabolism, amino acid and nucleotides concentration and their degradation products. Data thus obtained will be analyzed using Pathview, which is a R package, in order to determine which metabolic pathways are altered after OLT.

5.4.3 INFLAMMATORY CYTOKINE QUANTIFICATION.

Plasmatic cytokine will be monitored using commercially available arrays (MSD technology). V-PLEX Proinflammatory Panel 1 Human Kit will be used to quantitatively and multiplex measure a panel of 10 biomarkers associated with inflammatory response and immune system regulation such as IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL10, IL-12p70, IL-13, TNF-α. MSD V-PLEX technology allows the simultaneous quantification of 10 biomarkers in a small volume of human plasma.

5.4.4 SCRNASEQ

scRNAseq will be performed on whole PBMC to study modifications of RNA expression profile.

5.4.5 T-CELL PROLIFERATION

T-cell proliferation will be evaluated after CFSE labeling and anti-CD3 and anti-CD28 stimulation by flow cytometry. Whole PBMC will be defrost and cultured to evaluate the impact of immunosuppressive environment on T-cell proliferation.

5.4.6 SEAHORSE

T-cell mitochondrial respiration will be studied using the Seahorse technology (Agilent). After T-cell purification from defrost PBMC, Agilent Seahorse XF T Cell Metabolic Profiling Kit will be used to evaluate glycolytic and mitochondrial activity in T-cells.

5.5 OVERVIEW

Cf. Appendix «Study Design ».

6 END OF RESEARCH AND PARTICIPANT MANAGEMENT

6.1 END OF PARTICIPATION IN THE STUDY AND FOLLOW-UP OF EXPOSED PARTICIPANTS

6.1.1 END OF STUDY

End of study will be achieved at D28. After the end of study, there is no specific follow up or action performed for the subjects.

6.1.2 DISCONTINUATION OF PARTICIPATION OF A PERSON IN STUDY

Subjects can withdraw their consent and ask to withdraw from the study at any time and for whatever reason. Data prior to this withdrawal of consent will be collected unless the participant objects in writing.

The investigator can temporarily or permanently discontinue the study treatment for any reason which is in the best interest of the subject.

If a subject discontinues the study before its completion, the investigator must document the reasons as completely as possible.
A participant's exit from the study should not change anything in his medical care handling of his disease.
If a subject is lost to follow-up, the investigator will make every effort to recontact that person.

6.2 PRE-INCLUDED SUBJECTS NOT RANDOMISED

For subjects who signed the consent but finally not definitely included (randomised), only the reason for discontinuation of the enrollment process will be collected.
Fore more details, please refer to section 4.2

6.3 DISCONTINUATION OF PART OR OF THE ENTIRE STUDY

Unexpected events or new information pertaining to the product, in light of which the study objectives or clinical program probably would not be achieved, can lead the sponsor to terminate the study.

The sponsor reserves the right to interrupt the study at any time if it is found that the inclusion objectives are not met. In case of early permanent discontinuation of the study, the information will be sent by the sponsor within 15 days to the relevant country health agency and to the ethics committee or earlier according to local regulations.

6.4 SIMULTANEOUS PARTICIPATION IN OTHER RESEARCH AND EXCLUSION PERIOD

Subjects cannot be included in any other study from randomization to D28.

This research does not require an exclusion period during which the subject cannot participate in another clinical research protocol after the end of the study or after its premature termination.

6.5 COMPENSATION

Given :

- the study is based on the routine care handling,
- the study will essentially take place during one hospitalisation,
- the benefit/risk ratio is highly favourable,

no specific compensation is foreseen for this study.

6.6 REGISTRATION IN THE NATIONAL FILE OF PERSONS WHO ARE SUBJECTS IN BIOMEDICAL RESEARCH

There is no provision for registration in the national file of persons who lend themselves to research involving the human person.

7 TREATMENT(S) USED DURING THE STUDY

7.1 DESCRIPTION AND ADMINISTRATION

7.1.1 EXPERIMENTAL TREATMENT

7.1.1.1 Identification

The treatment is: **Human Albumin 20% 100ml**.

For this study, all proprietary medicinal product of Human Albumin 20% 100ml with a market authorisation are allowed. The Summary of Product Characteristic of Vialebex 200 g/L, solution for infusion, will be used as a reference.

7.1.1.2 Indication

Restoration and maintenance of circulating blood volume where volume deficiency has been demonstrated, and use of a colloid is appropriate.

7.1.1.3 Administration

Daily serum albumin level will be performed during the first 7 days following liver transplant in both groups.

Everyday, as soon as the result of albumin serum concentration is received, infusion of Human Albumin will be performed :

- From D0 to D5
  - when albumin serum concentration is at 30 g/L or below (treated group)
  - when albumin serum concentration is at 20 g/L or below (control group)
- D6 and D7
  - according to site current practice (all groups)

Subjects enrolled in the study will receive an intravenous infusion of 20% human albumin in 100 mL vials (20 g in 100 mL infusion bottle) in approximately 30–60 min.

The first dose will be administered, if needed, after randomization and will be infused by nursing personnel.

7.1.1.4 Dosage adjustment

The volume of Albumin solution prescribed is determined by the subjects' serum albumin level that morning based on China et al. (37), and Caironi et al. (14) and clinical experience. All clinicians could deviate from the suggested regimen, but were requested to document their reasons for doing so.

Dosage adjustment will follow daily serum albumin level. A calculator will be available to determine the exact amount of Albumin to infuse based on the formula below :

Determination of human albumin volume to infuse will be completed following the formula :

N= vials of 20%HA to infuse (20 g in 100 mL infusion bottle); PV= plasmatic volume; Ht=hematocrit (50%=0.5); IBW=ideal body weight (Ideal body weight is computed in men as 50 + (0.91 × [height in centimeters − 152.4]) and in women as 45.5 + (0.91 × [height in centimeters − 152.4]).

Plasmatic volume will be calculated as follow: 0.07 x [1-Ht]X[IBW]

To maintain plasmatic albumin level > 30 g/L: N (**whole number**) = ((30-[albu])xPV)/17

To maintain plasmatic albumin level > 20 g/L: N (**whole number**) = ((20-[albu])xPV)/17

For instance, to maintain plasmatic albumin level > 30g/L with PV 3.5 L, albumin level measured 22 g/L => N =1,65; thus N=2

7.1.1.5 Reference manual

For this study, all proprietary medicinal product of Human Albumin 20% 100ml are allowed. The Summary of Product Characteristic of Vialebex 200 g/L, solution for infusion, will be used as a reference.

7.1.2 ALLOWED ASSOCIATED TREATMENTS AND/OR PROCEDURES

No specific interactions of human albumin with other medicinal products are known. Every treatment will be authorized

No specific interactions of human albumin with other medicinal products are known. There is no unauthorized treatments.

7.2 EXPERIMENTAL DRUG(S) CIRCUIT(S)

7.2.1 GLOBAL TRACKING

7.2.1.1 Packaging and labeling

The investigational medicinal product is placed on the masrket as an authorised medicinal product. Therefore the labeling consists solely of the commercial labelling of the medicinal product. (Article 66 §1.b of Clinical Trials Regulation (EU) No 536/2014)

7.2.1.2 Manufacturing and distribution

The treatment units used for the research will be derived from commercial batches. Each site will source Human Albumin according to its current practice.

7.2.1.3 Stock management, resupply and dispensing

The treatment will be taken from the current stock of each pharmacy, labelled for the study. Units of Treatment will be subject of reimbursement by the health insurance funds in compliance with the conditions mentioned in III-1° of article L1121-16-1 of the French Public Health Code.

In case of shortage, the site will be allowed to temporarily suspend the inclusions.

The dispensing circuit will be carried out according to the practices of the sites and in compliance with the regulations relating to blood-derived medicinal products.

7.2.2 STORAGE CONDITIONS

The precautions for storage are those indicated in the summary of product characteristics and on the package leaflet.

7.3 METHODS FOR MONITORING TREATMENT COMPLIANCE

The dispensing and follow up will be performed in compliance with blood-derived medicinal products circuit.

The regulations relating to blood-derived medicinal products require that the treatments administered to be traced. The investigator will therefore provide the CRA with the documents required to verify compliance and the traceability of treatments.

The vials will be destroyed in ICU and shall not return to the pharmacy.

7.4 ALLOWED AND PROHIBITED TREATMENTS

7.4.1 ALLOWED TREATMENTS

No specific interactions of human albumin with other medicinal products are known. Every treatment will be authorized.

7.4.2 PROHIBITED TREATMENT(S)

No specific interactions of human albumin with other medicinal products are known. There is no unauthorized treatments.

7.4.3 EMERGENCY TREATMENT

Mild reactions such as flush, urticaria, fever, and nausea occur rarely. These reactions normally disappear rapidly when the infusion rate is slowed down or the infusion is stopped. Very rarely, severe reactions such as shock may occur. In these cases, the infusion should be stopped and an appropriate treatment should be initiated.

8 SAFETY EVALUATION

The definitions of the terms used in vigilance (adverse event, adverse effect, overdose, seriousness criteria, etc.) as well as the role of the sponsor in the analysis of serious adverse events and the transmission of safety information to the Competent Authorities are given in annex to the protocol.

8.1 EXPECTED ADVERSE EFFECTS

- Expected adverse effects related to the investigational medicinal drug (tested)

They are listed in the following reference document: Albumin Vialebex 200 g/L, solution for infusion

8.2 ADVERSE EVENTS NOTIFICATION (FROM INVESTIGATOR TO SPONSOR)

8.2.1 ADVERSE EVENTS TO BE NOTIFIED OR NOT BY THE INVESTIGATOR

Must be notified to the sponsor:

- ➔ Adverse events related (serious or non-serious) to the experimental treatment according to the investigator
- ➔ All causes of death
- ➔ All events (serious or non-serious) that investigators deem relevant to report to the Sponsor

8.2.2 NOTIFICATION PERIOD

The investigator must collect and notify all serious and non-serious adverse events (cited above) from randomization to D28.

Each adverse event will be followed until its complete resolution (stabilization at a level deemed acceptable by the investigator or return to the previous state) even if the participant has left the trial (follow-up notifications). In addition, whatever the time to onset after the end of the study, any serious adverse event likely to be due to the research must be notified to the sponsor when no cause other than the research can reasonably be attributed to it.

8.2.3 NOTIFICATION MODALITIES

8.2.3.1 All adverse events (all types)

Adverses events described section 8.2.1 will be reported in the eCRF on the "adverse event" page, specifying the date of occurrence, date of resolution, seriousness, description, intensity, causality and measures taken.

8.2.3.2 Serious adverse events

They will be transmitted, without delay, to the vigilance service of the Rennes University Hospital (by fax to 02-99-28-40-10 or email vigilance.essais@chu-rennes.fr).

The investigator must complete the SAE provided for this purpose and also attach to this form, whenever possible:

- copies of the hospitalization report, laboratory results, examinations results including relevant negative results
- possibly, a copy of the autopsy report;
- any other document it deems useful and relevant.

These documents will be pseudonymized and will bear the participant's identification number

8.3 MEDICATION ERRORS AND SPECIAL SITUATIONS NOTIFICATION (FROM INVESTIGATOR TO SPONSOR)

Medication errors or special situations are recorded in the case report form on the dedicated page.

If they have induced, possibly induced or could have induced a serious adverse event, they must be notified without delay to the sponsor via the SAE notification form.

9 DATA MANAGEMENT AND STATISTICS

9.1 RANDOMISATION

The randomisation ratio is 1:1.

The randomisation list will be produced by the statistician. The randomisation will be centralized by the department of Service de Pharmacologie and Centre d'Investigation Clinique - INSERM 1414 (University Hospital Rennes) and stratified by centre.

Randomization will be performed in the centres via a web interface (Ennov Clinical, Ennov Group, Paris, France). A guideline document to using this tool will be provided to investigators.

9.2 METHODS FOR DATA COLLECTION / CASE REPORT FORM

All information required by the study protocol must be recorded in the case report forms and an explanation must be provided for any missing data. Data must be collected progressively as obtained and recorded explicitly in the case report forms.

An electronic case report form (eCRF) will be made available and data entry will be completed in centres through a web interface (Ennov Clinical, Ennov, Paris (75), France). A guideline document to using this tool will be provided to investigators.

The interface between the CRA and the investigator thus will be promoted, making possible the collection and control of data at a distance. Consistency checks of data will be incorporated in electronic format. An audit function is incorporated in the eCRF thus making it possible to follow any change in study data. This function also makes it possible to clearly identify the person who made a change as well as the date. A justification possibly can be incorporated in comment.

Before the database lock, the e-CRF will be electronically signed by the Principal Investigator and an electronic copy will be sent to him/her for archiving. An electronic copy will be stored on the sponsor's server.

9.3 DESCRIPTION OF PLANNED STATISTICAL METHODS

Statistical analysis will be performed on all randomised and evaluated subjects (intention to treat analysis). It will be performed with SAS software (SAS Institute, Cary, NC, USA) or equivalent in the Biometrics Unit department of pharmacology of Rennes.

9.3.1 DESCRIPTIVE ANALYSIS

A first overall descriptive analysis and analysis by group will be performed. This consists of separate estimates, numbers and percentages for qualitative variables, means, standard error, medians and interquartile intervals for quantitative variables. The normal feature of the distribution of quantitative variables is checked.

9.3.2 COMPARISON OF GROUPS AT INCLUSION

Subjects' characteristics will be described in the two randomization groups at inclusion. Even if this is a randomized trial, comparison between groups will be performed in order to verify baseline comparability of the two groups. Student's t test or a Mann-Whitney test if necessary will be used to compare quantitative variables, and a Chi² or Fisher's exact test if necessary will be used to compare qualitative variables between two groups at inclusion

9.3.3 ANALYSIS OF THE PRIMARY CRITERIA AND OTHER CRITERIA

9.3.3.1 Analysis of the primary criteria

The incidence of AKI after OLT within 7 days after surgery will be compared between groups with the Chi² test.

9.3.3.2 Analysis of other criteria

Student's t test or a Mann-Whitney test if necessary will be used to compare quantitative variables. For qualitative criteria, a Chi² test will be used. For all these analysis, adjustments can be made in case of heterogeneity at inclusion.

9.3.3.3 Analysis of adverse events

Possible adverse events are coded according to the MedDRA classification and are the subject of a descriptive analysis.

9.4 PLANNED NUMBER OF PERSONS TO BE ENROLLED IN THE STUDY

Our hypothesis is that the human albumin perfusion during five days after OLT to achieve serum concentration above 30 g/l diminishes the acute kidney injury rate after liver transplantation from 45% to 30% . 164 subjects per group is required to detect a decrease in the rate of AKI from 45% to 30% after OLT when using albumin administration to achieve serum concentration above 30g/l, using a two-sided test with a type I error of 5% and a power of 80% (nTerim, V4.0, Statistical solutions Ltd, Cork, Ireland). A total of 400 subject will be randomized (200 per group) to take into account around 10% nonevaluable subjects or dropouts (consent withdrawal).

9.5 LEVEL OF STATISTICAL SIGNIFICANCE

All tests will be carried out with a 5% level of significance ($p<0.05$).

9.6 STATISTICAL CRITERIA FOR DISCONTINUATION OF STUDY

None.

9.7 METHOD OF MANAGEMENT OF MISSING, UNUSED OR INVALID DATA

Missing data will not be replaced. Mixed models can be used in analysis of repeated data.

9.8 MANAGEMENT OF CHANGES MADE TO PLAN OF ANALYSIS OF INITIAL STRATEGY

Changes compared to the initial strategy can be made depending on conduct of the study and repetition in the statistical analysis plan will be written just prior to locking of the database.

9.9 CHOICE OF PERSONS TO INCLUDE IN THE ANALYSIS

This trial is an intention to treat study, that is all randomised and evaluated subjects will be analysed.

10 ADMINISTRATIVE AND REGULATORY REQUIREMENTS

10.1 STUDY FEASIBILITY

We have performed a retrospective analysis supporting our hypothesis (association between albumin and AKI). Eligibility criteria and study design will not hinder enrollment expectations. With 9 centers (almost 750 LT/year) enrollment goals will be achieved. Study plan does not conflict with currently accepted practice.

The study is coordinated at the logistical, financial and regulatory level by the Research and Innovation Department of the University Hospital of Rennes; certified ISO9001 for its clinical research activities. The monitoring of the study will be ensured by a sponsor CRA of the the CHU of Rennes.

Additional costs, notably for personal time, are expected.

Regarding immunomonitoring, our team (SITI, Suivi Immunologique des Thérapies Innovantes), certified ISO 9001, will perform analysis. The team conducted immunomonitoring study in different RCT (NCT04404426; NCT03372174; NCT04513288; NCT02864017)

10.2 ASSOCIATE INVESTIGATORS AND RESEARCH MONITORING

10.2.1 ASSOCIATE INVESTIGATORS

Cf. Appendix «List of investigators and sites ».

10.3 FUNDING AND INSURANCE

10.3.1 FUNDINGS

This study was financed by the Clinical Research Hospital Program (PHRC) and the Research and Innovation Department (DRI) of the University Hospital of Rennes.

10.3.2 INSURANCE

The Sponsor will take out, for the duration of the study, insurance covering the sponsors civil responsibility as well as that of all doctors/professional of health involved in conduct of the study. They will also insure the total compensation of all harmful consequences of the study for persons who are subjects in it and their heirs, except when evidence is provided that the harm is not causally related to a fault of the sponsor or that of any participant, without it being possible to oppose the intervention of a third party or the voluntary withdrawal of a person who had initially consented to be a subject in the study.

10.4 ETHICS COMMITTEE

The sponsor submits the study protocol, the information letter and the consent form of the study to an independent ethics committee (IEC), in accordance with article L 1121-1 of the the French public health code, for a favorable opinion and confirmation of the qualification of the research, before any implementation of the research.
Notification of the IEC's favourable opinion is sent to the study sponsor and to the Competent Authority.

10.5 COMPETENT AUTHORITY

The sponsor submits a request for authorization for the study to the local competent authority; the National Health Regulatory Agency (ANSM).

10.6 SUBSTANTIAL MODIFICATIONS

Any substantial modification to the study protocol should be notified to the competent authority and the ethics committee in order to verify that the proposed modifications do not alter at any time the safeguards provided to the persons undergoing the research.

In case of a substantial change made to the study protocol by the investigator, it will be approved by the sponsor. The latter must obtain prior to start of study a favourable opinion from the ethics committee and authorization from the relevant health authority in the setting of their respective competence.

Additional consent from persons participating in the study will be collected if necessary.

10.7 PROCESSING OF PERSONAL DATA - CNIL

This study falls within the scope of the "Reference Methodology" (MR-001) in application of the provisions of article 54 paragraph 5 of french law no. 78-17 of 6 January 1978, as amended, relating to information technology, files and freedoms. This change was approved by decision dated 5 January 2006. The CHU of Rennes, promoter of the study, has signed a commitment to comply with this "Reference Methodology"

10.8 RIGHT OF ACCESS TO DATA AND SOURCE DOCUMENTS

10.8.1 ACCESS TO DATA

In compliance with GCP:

- the sponsor is in charge of obtaining the agreement of all parties involved in the study to ensure direct access to all sites of study conduct, source data, source documents and reports with the aim of checking quality and of an audit by the sponsor,
- investigators will make available to persons in charge of monitoring, checking of quality or audit of the study documents and individual data which are strictly necessary for such checking, in compliance with legislative and regulatory conditions in force (articles L.1121-3 and R.5121-13 of the French public health code).

10.8.2 DATA CONFIDENTIALITY

In compliance with conditions concerning confidentiality of data to which persons in charge of quality control of the biomedical study have access (article L.1121-3 of the French public health code), in compliance with conditions pertaining to confidentiality of information in particular concerning the type of investigational medicinal products, the tests, persons who are subjects in the study and results obtained (article R. 5121-13 of the French public health code), persons having direct access will take all necessary precautions with the aim of ensuring confidentiality of information pertaining to the investigational medicinal products, tests, the persons who are subjects in the study and in particular concerning their identity as well as that of results obtained.

These persons, in the same capacity as the investigators themselves, are subject to professional secrecy (according to conditions defined by articles 226-13 and 226-14 of the French penal code).

During the study or at its end, data collected on persons who are subjects in the study and forwarded to the sponsor by the investigators (or all other specialised participants) will be made anonymous (deletion of subjects' names).

They must not in any event clearly reveal the names of the persons concerned or their address.

Only the first letter of the surname and of the first name of the subject are recorded, together with the code number specific for the study indicating the order of inclusion of subjects.

The sponsor will make certain that each person who is a subject in the study has provided his or her written agreement for access to his/her personal individual data and strictly necessary for quality control of the study.

10.9 REPORTING OF DEVIATIONS AND SERIOUS BREACHES

10.9.1 DEVIATIONS

Deviations from clinical trial protocols and GCP may occur during clinical trials. In the majority of cases, these are technical deviations that do not result in harm to the trial subjects and do not significantly

affect the scientific value of the trial results. These cases should be documented (for example, in the trial report form or in the main trial file) so that appropriate corrective and preventive measures can be taken.

10.9.2 SERIOUS BREACHES

A 'serious breach' means a breach likely to affect to a significant degree the safety and rights of a subject or the reliability and robustness of the data generated in the clinical trial. The sponsor notify the Member States concerned about a serious breach of this Regulation or of the version of the protocol applicable at the time of the breach through the EU portal without undue delay but not later than seven days of becoming aware of that breach

10.10 METHODS OF MONITORING THE CONDUCT OF THE CLINICAL TRIAL

10.10.1 DATA AND SAFETY MONITORING BOARD

The use of albumin in the two strategies compared in the HALT study falls within the scope of the indications set out in the Summaries of Product Characteristics for the different albumin specialities used. It is considered that there is now sufficient experience with albumin used in liver transplantation for the creation of an Independent Monitoring Committee to be considered superfluous.

10.10.2 COMMITTEE FOR EVALUATION OF INFECTIONS AND NEUROTOXICITIES

Two categories of events will be validated: infections and neurotoxicities.
The committee will be composed of :

- Intensivist
- Transplant physician/surgeon
- Infections (infection validation only)
- Neurologist (validation of neurotoxicities only)

The relevant documents will be uploaded by the site to the eCRF. A dedicated section of the eCRF will allow each practitioner to validate or not each case. In the event of conflicting opinions between practioners, meetings will be organized to reach a consensus.
In case of infection, the decisions will be performed base on the appendice « Criteria used for defining the sites of infection »

The investigator and associated persons agree to accept any quality assurance audits performed by the study sponsor and any inspections performed by the competent authority. All data, documents

and reports may be subject to regulatory audits and inspections, without prejudice to medical confidentiality.

10.10.3 MONITORING

A Clinical Research Associate (CRA) designated by the sponsor will ensure proper conduct of the study and the quality of data collected, in agreement with the Standard Operating Procedures (SOP) applied in the CHU of Rennes and in compliance with Good Clinical Practice (GCP) as well as legislative and regulatory conditions in force.

The investigator and members of his team agree to make themselves available at Quality Control visits carried out at regular intervals by the Clinical Research Associate. At these visits the following items will be reviewed:

- informed consent,
- compliance with study protocol and procedures defined in it,
- quality of data collected in the case report forms: accuracy, missing data, consistency of data with "source" documents (medical dossiers, appointment diaries, originals of laboratory test results, etc.),
- management of possible products.

10.11 NOTIFICATION OF THE START OF A CLINICAL TRIAL AND OF THE END OF THE RECRUITMENT OF SUBJECTS

The sponsor will notify, through the EU portal, each Member State concerned :

- of the start of a clinical trial (within 15 days)
- of the first visit of the first subject (within 15 days)
- of the end of the recruitment of subjects (within 15 days)
- of the end of the clinical trial (within 15 days)

10.12 RESULTS OF THE CLINICAL TRIAL

Irrespective of the outcome of a clinical trial, within one year from the end of a clinical trial in all Member States concerned, the sponsor will submit to the EU database a summary of the results of the clinical trial. The content of this summary is defined in the corresponding regulations.

It shall be accompanied by a summary written in a manner that is understandable to laypersons. The content of this summary is defined in the corresponding regulations.

10.13 RULES PERTAINING TO PUBLICATION

Scientific presentations and reports corresponding to the study will be written under the responsibility of the coordinating investigator of the study with the agreement of the responsible investigators.
Rules on publication will follow international recommendations (N Engl J Med, 1997; 336:309-315).
The study will be recorded on a freely accessible website (Clinical Trials) prior to inclusion of the first subject in the study.
First author Pauline Houssel, second author Bruno Laviolle, and last author Jean-Marc Tadié. The co-authors of the report and of publications will be the investigators and clinicians involved, on a pro rata basis of their contribution in the study, as well as the biostatistician and associated researchers.

10.14 COMMUNICATION OF STUDY RESULTS

Any external communication must be authorized by the sponsor before distribution, submission for publication, etc.
Any first publication using study data must be an originator publication.
All communications must include the NCT/other database number, EU number, funding, sponsor's name and logo.

10.15 Quality control, audit and inspection

The investigators and associated persons agree to accept any quality assurance audits carried out by the study sponsor and any inspections carried out by the competent authority. All data, documents and reports may be the subject of audits and regulatory inspections, without the right to invoke medical confidentiality.

10.16 ARCHIVING

Study documents will be kept by the sponsor and the departments involved in the research for at least 40 years after the end of the study or its early termination.
Source documents are defined as any original document or object that can be used to prove the existence or accuracy of a data or fact recorded during the clinical study. They will be kept for 40 years by the investigator or by the hospital if it is a hospital medical record
The following documents will be kept in the respective departments until the end of the period of practical utility.
These documents are:

- The study protocol and its annexes, and possible amendments,
- The original signed informed information and consent forms,

- Individual data (authenticated copies of raw data),
- Monitoring documents,
- Statistical analyses,
- The study final report.

Archived documents cannot be moved or destroyed without agreement of the sponsor. All data, all documents and reports can be subjected to an audit or inspection.

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12 APPENDIX

REFERENCE	DESCRIPTION
ADVERSE EVENTS	AE : Adverse events : Definitions and sponsor's role
BANFF	Banff Schema for Grading Liver Allograft Rejection: An International Consensus Document
DISCHARGE	Criteria to determine readiness for hospital discharge following liver transplantation
HYPOALBUMINEMIA	Hypoalbuminemia following orthotopic liver transplantation is associated with acute kidney injury and tacrolimus associated neurological events in Intensive Care Unit
INFECTION	Criteria used for defining the sites of infection
INVESTIGATORS	List of investigators and sites
KDIGO	KDIGO Criteria for Acute Kindey Injury after Orthotopic Liver Transplantation
NEUROLOGICAL COMPLICATIONS	Neurological Complications Occurring After Liver Transplantation: Role of Risk Factors, Hepatic Encephalopathy, and Acute (on Chronic) Brain Injury
STUDY DESIGN	Design of the study