Trial Protocol

Granulocyte colony stimulating factor (G-CSF) to treat acute-on-chronic liver failure: A multicentre randomized trial

GRAFT-Trial

Gefördert von der Deutschen Forschungsgemeinschaft
Geschäftszeichen: EN 1100/1-1

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Table of Contents

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Synopsis

Title of the trial	Granulocyte colony stimulating factor (G-CSF) to treat acute-on-chronic liver failure: A multicenter randomized trial
Acronym	GRAFT-Trial
Indication	Acute-on-chronic liver failure (ACLF)
Primary goal of the trial / primary end point	Primary efficacy endpoint: Transplant-free survival up to 90 days (death or transplant count as events)
Secondary goals of the trial / secondary end points	Key secondary endpoint(s): • Overall survival at 360 days • Transplant-free survival at 360 days • Complications of ACLF within 90 days/within 360 days (hepatorenal syndrome, variceal bleeding, ascites, hepatic encephalopathy) • Infections within 90 days/within 360 days (proven infection necessitating systemic use of antibiotics) • Liver function during the course of treatment and follow-up (MELD-Score, Child-Pugh-Score) • Duration of initial hospital stay Assessment of safety: In addition to the complications of ACLF listed above, further AEs and SAEs will be assessed. Laboratory values reflecting liver function as well as infection related parameters will be monitored during the course of treatment and follow-up.
Trial design	Prospective, open, randomized, controlled multicenter trial
Trial population	Key inclusion criteria: Acute-on-chronic liver failure according to the criteria defined by the CANONIC study [Moreau 2013] / age >= 18 years / Informed consent Key exclusion criteria: Prior not curatively treated or active malignancies / sickle cell disease / septic shock, defined by the following symptom complex: bacteraemia AND SIRS AND shock / WBC-count of **>** 50 x 10^9/L / known HIV infection / known intolerance to filgrastim / pregnancy, lactation or insufficient contraception / participation in other interventional clinical trials
Sample size	*To be assessed for eligibility:* *n = 1200* To be assigned to the trial: **n = 292** To be analysed: **n = 262**
Therapy	Experimental intervention: Application of G-CSF (Filgrastim) in combination with standard care of acute-on-chronic liver failure (G-CSF subcutaneously, on day 0-4, then every 3rd day over 26 days (days 7, 10, 13, 16, 19, 22, 25) = 12 doses) G-CSF doses should be guided by the body weight using a cut off value of 70 kg (≤ 70kg 30 Mio IU G-CSF, > 70 kg

	48 Mio IU G-CSF) Control intervention: Standard care of acute-on-chronic liver failure Follow-up per patient: 1 year (baseline, 1, 4, 7, 14, 28, 90, 180, 360 days) Duration of intervention per patient: 26 days Experimental and/or control off-label or on-label in Germany: experimental off-label
Biometry	Efficacy: Two-sided superiority test on equality of transplant-free survival up to 90 days Description of the primary efficacy analysis and population: Confirmatory analysis of the primary endpoint will be performed using Cox regression adjusting for ACLF grade, following the intent-to-treat principle and based on the full analysis set. One interim analysis is scheduled for early superiority when 50 % of the patients have 90 day follow-up (for details see section 8.7). Safety: Descriptive analysis of (serious) adverse reactions/events and lab values. Annual safety reports. Secondary endpoint(s): Analysed in a descriptive manner by appropriate methods depending on the scale of the endpoint. Predictors of treatment outcome will be investigated by multivariate analyses.
Trial Duration	First patient in to last patient out (months): 48 Duration of the entire trial (months) including preparation and analysis: 60 Recruitment period (months): 36

Schedule of Assessments and Procedures

The following table shows the proposed visit schedule, together with all corresponding assessments.

visit / day	**Baseline** -2 bis 0	**V1** 1 [a]	**V2** 4[b]	**V3** 7[b]	**V4** 14[c]	**V5** 28[c]	**V6** 90[d]	**V7** 180[e]	**V8** 360[e]
Eligibility criteria	x								
Informed consent	x								
ACLF grade	x			x	x	x			
Randomisation	x								
Concomitant diseases	x								
Clinical assessment									
Vital signs[1]	x	x	x	x	x	x	x	x	x
Blood gas analysis[2] **or** saturation of peripheral oxygen (PaO_2 or SpO_2) **and** fraction of inspired oxygen (FiO_2)	x			x	x	x	x	x	x
ACLF Complications [3]	x	x	x	x	x	x	x	x	x
Drug accountability	x	x	x	x	x	x			
Adverse events	x	x	x	x	x	x	(x)	(x)	(x)
Concomitant medication	x	x	x	x	x	x			
Local laboratory tests									
Liver function test [4]	x	x	x	x	x	x	x	x	x
Chemistry [5]	x	x	x	x	x	x	x	x	x
Serum creatinine	x	x	x	x	x	x	x	x	x
C reactive protein	x	x	x	x	x	x	x	x	x
Procalcitonin	x	x	x	x	x	x			
Alpha fetoprotein	x		x						
Haematology [6]	x	x	x	x	x	x	x	x	x
Urinalysis [7]	x								
β-hCG test	x								
Abdominal ultrasound	x				x	x	x	x	x
Duplex ultrasound (liver vessels)[8]	x				x				x
G-CSF injection (**only experimental arm**)[9]	x	x	x	x					
Blood sampling for scientic sub-projects [10]	x	x	x	x	x	x	x	x	x

[1] arterial blood pressure, heart rate, temperature, respiratory rate, and body weight at baseline

[2] capillary or arterial

[3] hepatorenal syndrome, variceal bleeding, ascites, hepatic encephalopathy

[4] AST, ALT, gamma-GT, total bilirubin, albumin, alkaline phosphatase, INR

[5] sodium, potassium, urea

[6] erythrocytes, total leukocytes with differential, platelets, haemoglobin, haematocrit

[7] urinary sodium excretion, urine status, urine sediment test

[8] for the exclusion of a thrombosis

[9] G-CSF injections: the first 5 days (0,1,2,3,4), then every 3rd day over 26 days (days 7, 10, 13, 16, 19, 22, 25)

[10] optional

[a] + 1 day

[b] ± 1 days

[c] ± 2 days

[d] ± 7 days

[e] ± 14 days

Table 1: Visit schedule

Flow Chart

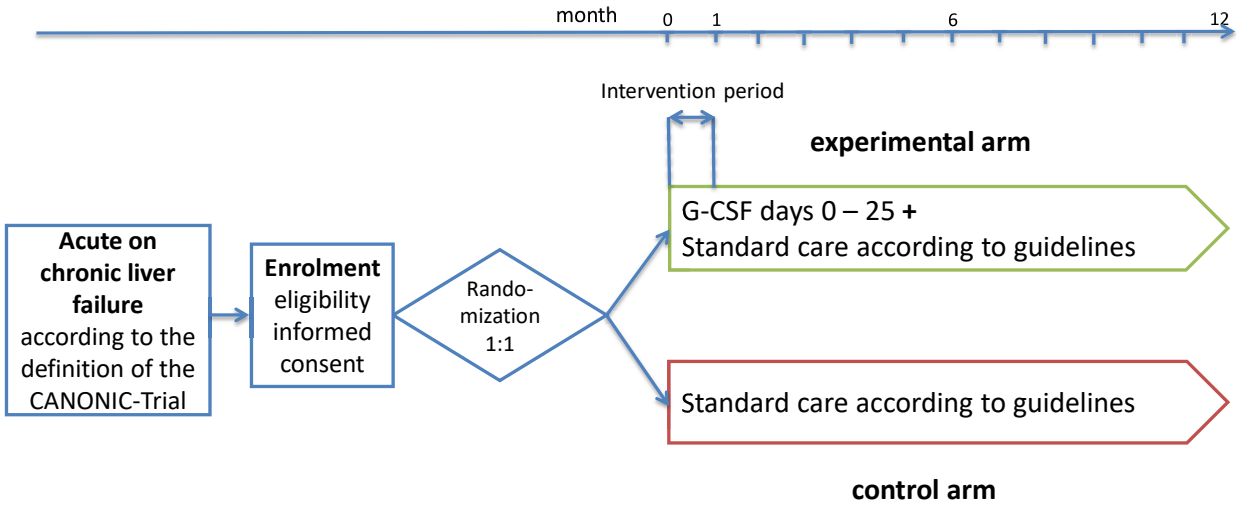

Figure 1: Scheme of intervention and data analysis

1 RATIONALE

1.1 Medical Background

The acute-on-chronic liver failure (ACLF) is defined by a rapid deterioration of liver function on top of an underlying compensated chronic liver disease. Precipitating events include variceal bleeding, infection, alcohol consumption, viral hepatitis or drug-induced toxicity [Laleman 2011, Sarin 2009]. The prognosis is poor, reflected by a 28-day mortality of 22 – 77 % and a 3-months mortality of up to 80 % [e.g. Moreau 2013]. Organ failure is one of the main clinical features of this entity. However, pathophysiological aspects leading to an acute deterioration of liver function as well as organ failure are not well understood. In patients with chronic liver diseases an immune dysfunction is believed to be the main driver for infectious complications that further aggravate the inflammatory response und subsequently lead to ACLF (Jalan R et al. J Hepatol 2012;57:1336-1348). Orthotopic liver transplantation (OLT) is the only curative therapeutic option in these patients. However, due to contraindications and shortage of donor organs, only few patients may benefit from this effective approach. Alternative treatment options such as extracorporeal artificial liver support failed to improve patients` outcome [Bañares 2013, Kribben 2012]. Thus, in the majority of patients, treatment options are limited to the elimination of precipitating events and the control of complications such as hepatorenal syndrome, ascites, encephalopathy, circulatory dysfunction and coagulopathy. However, two recent small, randomized trials from Asia reported an impressive effect of G-CSF on liver function and survival in patients with ACLF [Garg 2012, Duan 2013].

1.2 Rationale

1.2.1 Evidence

The loss of functional hepatocytes in ACLF is mainly triggered by the immune system. Key players for tissue damage are monocytes, macrophages and effector CD8 T-cells that are presumably activated by cytokine bursts [Olson 2011, Maiwal 2013]. In addition, antigen presenting dendritic cells, which are potent immune-modulators maintaining the equilibrium between pro- and anti-inflammatory factors in liver tissue and peripheral blood, are significantly dysfunctional in ACLF patients [Maiwal 2013]. This leads to an activation of IFNγ producing cytotoxic T-cells causing further liver damage [Maiwal 2013]. In contrast, decreased activity of phagocytic cells, mainly neutrophils, impairs defence against infections and potentially leads to the systemic inflammatory response syndrome and in consequence to multi-organ failure [Moreau 2013, Jalan 2012, Khanam 2014]. Thus, a reasonable therapeutic approach should aim at a reduction of the hepatotoxic activity of the immune system, the improvement of the hepatic repair capacity as well as the restoration of the immune function.

Endogenous hepatic regeneration processes are complex and mainly consist of the replication of mature hepatocytes, the activation and differentiation of hepatic progenitor cells, as well as the recruitment of bone marrow derived stem cells [Maiwal 2013, Rhiele 2011, Schmelzle 2013]. Hepatic stem cells play an important role in the proliferative capacity of the liver as they can differentiate into hepatocytes and bile duct epithelial cells.

Stem cells can also be mobilized from the bone marrow into the circulation [Maiwal 2013, Wan 2013, Schmelzle 2013]. These hematopoietic stem cells can replace damaged endothelial cells, serve as endothelial precursors and remodel hepatic repair capacities [Grompe 2003, Bird 2008]. The systemic administration of bone marrow derived stem cells (BMSC) improved liver function (i.e. decreasing bilirubin, increasing prothrombin time) in models of surgical (i.e. liver resection) and pharmacological liver injuries [Grompe 2003, Li 2010]. The administration of BMSC directly via the portal vein or hepatic artery in pre-clinical

as well as phase I clinical trials accelerated early liver regeneration [Schulte am Esch 2012, Levicar 2008, Salama 2010, Nakamura 2014]. In a randomized controlled trial in 77 patients with decompensated hepatitis B cirrhosis autologous bone marrow stem cell transplantation significantly promoted tissue repair after 4 weeks [Li 2014]. However, therapeutic use of BMSC is hampered by regulatory issues, technical difficulties in collecting and preparing stem cells, and not at least costs.

G-CSF increases the amount of circulating bone marrow derived stem cells. It is widely used for harvesting peripheral blood stem cells for transplantation, both in healthy stem cell donors and in patients scheduled for an auto-transplant. In addition, G-CSF accelerates recovery of neutrophils after chemotherapy. Administration by subcutaneous injection is easy and safe. The G-CSF receptor is expressed on a vast amount of cell-types including monocytes/macrophages, endothelial cells, lymphocytes and natural killer cells. Consequently, G-CSF not only stimulates stem cell release from the bone marrow, but also has immune-modulatory effects: It promotes neutrophil maturation, phagocytosis and bactericidal activity and inhibits pro-inflammatory cytokine production, increases the amount of tolerant dendritic cells, and reduces IFN-production in lymphocytes [Khanam 2014, Martins 2010]. The interplay of these cell lines restores the function of the host immune systems avoiding further immune related liver damage but also supporting defence mechanisms against infections [Martins 2010]. Endogenous G-CSF levels are increased during regeneration in patients with liver failure, but its effect can be further supported by external G-CSF administration [Hamilton 2008, Matsumoto 2013, Hosing 2012].

In rat models of liver failure, G-CSF mobilised hematopoietic stem cells could be detected in liver tissue. Serum markers of liver injury and liver histology revealed a decrease in liver inflammation and an increase in hepatocyte mitotic activity after G-CSF administration. Untreated rodents had a low survival rate of 25 % at 18 hours after induction of acute liver failure. In contrast, animals with identical liver injury but G-CSF administration showed a survival rate of 60 % ($p < 0.001$) [Theocharis 2003, Qujeq 2013, Mark 2010, Zhang 2011].

In phase I trials in humans safety and mobilisation efficacy of G-CSF was demonstrated in patients with compensated liver cirrhosis [Di Campli 2007, Lorenzini 2008, Gaia 2006]. The amount of hepatic growth factor, intrahepatic progenitor cells and proliferative activity increased, strongly suggesting that G-CSF indeed has an impact on liver regenerative capacity [Spahr 2008].

A recently published randomized study from India in 47 patients with ACLF reported a striking improvement of short-term survival (60 day-survival 26 % vs. 66 %) and liver function (median reduction of MELD-score about 15.3 % compared with an increase of 11.7 % in controls) after G-CSF administration [Garg 2012]. A significant reduction of the frequency of infectious complications as well as hepatorenal syndrome and multi-organ failure (SOFA-Score) was also observed [Garg 2012]. A second randomized study from China in 55 patients with hepatitis B virus (HBV)-associated ACLF, confirmed the findings from the Indian study, reporting a 90 day-survival of 48 % in the G-CSF-group as compared to 21 % in the control group [Duan 2013].

Therefore, we hypothesize that G-CSF is a safe, easy available and effective treatment option for patients with liver failure.

Patients will be randomized 1:1 either to standard medical care plus G-CSF (Filgrastim) or standard medical care alone. After recovery from the first ACLF-episode, any subsequent ACLF-episode will be treated with standard medical care, irrespective of the randomization arm in order to evaluate the potential long-term consequences of G-CSF administration. Placebo control is neither feasible nor ethically justifiable in our study setting. Blinded treatment may lead to misinterpretation of a G-CSF associated white blood cell count (WBC) increase being a consequence of an infectious complication. In addition, G-CSF is often associated with non-serious side effects like bone pain and irritation of the injection site which also hampers blinding of the treatment arm. As an increase in WBC may not be used

as marker of infection in G-CSF-treated patients, SIRS-Criteria (except for leukocytosis), C-reactive protein (CRP), procalcitonin (PCT)-levels and if required culture based microbiological diagnostics will be used as standard screening tools for infectious complications throughout the trial period (for both treatment groups). Delayed diagnosis of infectious complications is not expected, as CRP and procalcitonin-levels are reliable markers for infections in liver cirrhosis [Papp 2012; Lazzarotto 2013]. Twelve doses of G-CSF will be administered subcutaneously on day 0-4, then every 3rd day over 26 days (days 7, 10, 13, 16, 19, 22, 25). This dose regimen corresponds to that used by Garg et al. [Garg 2012] who could show a significant survival improvement indicating that this regime is effective in the clinical setting of acute-on-chronic liver failure. Although a phase I trial in patients with compensated cirrhosis demonstrated a dose dependent effect on mobilization of CD34+ stem cells reaching its optimum with 10-15 µg/kg [Lorenzini 2008] other trials could show an effective mobilization in similar cohorts using 5µ g/kg [Di Campli 2007, Gaia 2006]. As the mobilization effect peaks not until day 4-7 after treatment initiation [Greenbaum 2011] an extension of the treatment period up to 26 days seems to be reasonable to prolong the positive effect on the hepatic repair capacity [Greenbaum 2011].

1.2.2 Need for a trial

ACLF is a condition with dismal prognosis, for which apart from OLT no therapeutic options exist. A treatment approach that helps to recover from this acute intercurrent event and hereby improves the very poor short-term survival would be of major clinical importance. Stem cells play a major role in the process of hepatic regeneration. Their mobilization from the bone marrow due to G-CSF in patients with liver failure could be demonstrated in several phase I/II trials. Although an impact on the hepatic regenerative capacity was obvious, the results for clinical endpoints like patients´ survival and liver function were controversial [Di Campli 2007, Lorenzini 2008, Gaia 2006, Spahr 2008]. This was most likely due to the fact, that the observation period was limited and the power was inadequate for these endpoints. Recently two small randomized trials have shown the potential of G-CSF in this patient population markedly improving patients´ outcome [Garg 2012, Duan 2013]. However, these results need to be confirmed in a large multicenter trial.

1.3 Risk-Benefit Considerations

Since the prognosis of ACLF is still poor new therapeutic options are needed. Stem and immune cells have a considerable impact on both, the mechanism leading to ACLF such as infections as well as on liver regeneration itself. G-CSF is capable to safely mobilize hematopoietic stem and immune cells in cirrhotics [Di Campli 2007, Lorenzini 2008, Gaia 2006]. But as first phase I/II trials were inadequately designed for clinical endpoints the impact on patients´ outcome was controversial [Di Campli 2007, Lorenzini 2008, Gaia 2006, Spahr 2008]. However, in two small randomized trials the administration of G-CSF showed a beneficial effect on the outcome after ACLF [Garg 2012, Duan 2013]. G-CSF is widely used for treatment of neutropenia and mobilization of hematopoietic stem cells in donors for stem cell transplantation. The safety profile is well known, with minor side effects like headache, fever or bone pain. Patients at risk for seldom, but major side effects of G-CSF (stimulation of malign cells or crisis of sickle cell disease) are excluded. Splenic rupture is another rare complication that can occur under the treatment of G-CSF. Patients with abdominal pain in the left upper quadrant or left shoulder should be immediately screened for splenic bleeding by perfoming an ultrasound. However, there are no reliable data about a critical spleen size that increases the risk for splenic rupture. Therefore we do not exclude patients with splenomegaly but encourage physicians to screen for clinical sign of splenic complications. In addition, none of the trials, which investigated G-CSF in ACLF or in other liver diseases, reported any serious safety issues. In conclusion, G-CSF seems to be a safe and potentially beneficial treatment option in ACLF. However, in the unlikely case of a serious adverse event deemed possibly, probably, or definitely related to G-CSF treatment study medication will be

stopped. In case of a WBC count >70 x 10^9/L under G-CSF treatment, drug administration will be discontinued in the individual patient. Treatment can be resumed if WBC count recedes 50 x 10^9/L.

All patients will receive best standard care, including the option for liver transplantation. It should be mentioned here that patients with ACLF are at high risk to develop sepsis. Increased white blood cell count is one indicator for early detection of sepsis. This indicator cannot be interpreted in the G-CSF treated patients. Therefore, SIRS-Criteria (except for leukocytosis), CRP- and PCT-levels and if required microbiological diagnostics will be used as standard screening tools for infections in this trial, in order to allow for early detection of sepsis.

No additional invasive measures will be performed.

ACLF treatment with G-CSF is an off-label indication and until now not recommended by national or international guidelines. Although recent publications clearly suggest a benefit these results must be confirmed by multicenter randomized trials and larger sample sizes. There are no ethical concerns regarding the control arm, as guideline-oriented treatment is applied.

2 OBJECTIVES

2.1 Primary Objective

From the clinical point of view, overall survival and transplant-free survival are the most relevant outcome measures. Thus, the primary endpoint of the study is **transplant-free survival up to 90 days, with death and liver transplantation (OLT) counting as event**. Due to shortage of donor organs in Germany, only a limited number of patients and mostly only those with a very poor prognosis as calculated by the MELD score may receive an organ. Therefore, in the context of our trial, OLT is regarded as treatment failure. The time horizon of 90 days has been chosen as it best reflects the short-term prognosis of this patient population, and is also the time frame, which may be predicted by the MELD score and the grade of ACLF [Moreau 2013]. Patients surviving the first ACLF episode are at risk for further ACLF episodes during follow-up. These further ACLF episodes constitute a source of variance on long-term outcome in both treatment groups. However, overall survival and transplant-free survival time will be assessed for the complete follow-up period as secondary endpoints. Unbiased evaluation is ensured since all further ALCF periods are treated by standard care only.

2.2 Secondary Objectives

Beside overall survival and transplant-free survival time until the end of follow-up (see above) the following secondary endpoints will be evaluated:

- Complications of ACLF (hepatorenal syndrome (HRS), variceal bleeding, ascites, hepatic encephalopathy (HE)) within 90 days/within 360 days.
- Infections within 90 days/within 360 days (proven infection necessitating systemic use of antibiotics)
- Liver function during the course of treatment and follow-up (MELD-Score, Child-Pugh-Score)
- Duration of the initial hospital stay

These further secondary endpoints have been chosen in order to better understand the impact of G-CSF administration on liver function and on the development of complications

during the course of ACLF, especially the rate of infectious complications. The time frame 90 days reflects the treatment of the initial ACLF event, while 360 days analysis reflects long-term effects.

In a subgroup of patients, differential cell analysis will be performed (see chapter 9).

3 TRIAL DESIGN AND DESCRIPTION

3.1 Trial Design

This is a prospective, randomized, controlled, open-label 2-armed multicenter study.

Patients will be randomized 1:1 either to standard medical care plus G-CSF (Filgrastim) or standard medical care alone. After recovery from the first ACLF-episode, any subsequent ACLF-episode will be treated with standard medical care, irrespective of the randomization arm in order to evaluate the potential long-term consequences of G-CSF administration.

3.2 Requirements at the Trial Sites regarding Personnel and Equipment

The common qualification criteria required by ICH-GCP and the German drug law will be assessed by the ethics committees involved before start of the trial.

3.2.1 Qualification of investigator/ deputy and medical staff in the study team (for German trial sites only)

The coordinating investigator in multicenter trials and the deputy are licenced to practice medicine, are medical specialists and have at least two years work experience in treatment of patients with *acute-on-chronic liver failure*. They have theoretical and practical experience in conducting clinical trials. Their qualification is defined as follows:

- Documented proof of at least two years experience in conducting clinical trials after August 2004 (12. Amendment of German Drug Law) incl. proof of GCP training

AND

- Updates of GCP knowledge and revisions of German Drug Law every two to three years

Investigator and deputy in the participating centres are licensed to practice medicine, are medical specialists and have experience in treatment of patients with *acute-on-chronic liver failure*. They have theoretical and practical experience in conducting clinical trials.They have theoretical and practical experience in conducting clinical trials. Their qualification is defined as follows

- Documented proof of the conduct of several clinical trials after August 2004 (12. Amendment of German Drug Law) incl. proof of GCP training

AND

- Updates of GCP knowledge and revisions of German Drug Law every two to three years

The Investigator is responsible for selecting and assembling the study team members (especially the medical staff) according to the requirements of this trial protocol. Furthermore, the investigator is responsible for training and supervision of the study team and providing all necessary information. This has to be documented.

Medical staff is licenced to practice and has at least theoretical experience in conducting clinical trials. The qualification is defined as follows

- Certification of successful participation in an investigator course incl. GCP training
  OR
- Documented proof of conducting clinical trials after August 2004 (12. Amendment of German Drug Law) incl. proof of GCP training

AND in addition

- Updates of GCP knowledge and revisions of German Drug Law every two to three years.

The staff at the sites will be trained for the eCRF data entry, query management and trial-specific procedures.

3.2.2 Essential technical equipment at the trial sites and involvement of other facilities in the trial

Specific requirements at study centres will include:

- Trained personnel for sampling, preparation and shipment of blood specimen (including infectious samples) for analysis at local laboratory
- Refrigerators for short-term storage of lab specimen
- PC for electronic data entry (electronic CRF)

3.3 Trial Sites and Number of Trial Subjects

The study is planned to be conducted in about twenty study centres in Germany.

The aim is to include a total number of 262 patients evaluable for the primary analysis. Assuming a drop-out rate of about 10 %, a total of 292 patients are to be randomized.

We expect that 1.200 patients will be assessed for eligibility.

3.4 Expected Duration of Trial

First patient in to last patient out (months): 48

Recruitment period (months): 36

Follow-up per patient (months): 12

Duration of intervention per patient: 26 days

Duration of the entire trial, including preparation and analysis (months): 60

The trial formally starts with the randomization of the first patient (FPI = first patient in), and the formal end of the study is the last visit of the last patient included (LPO = last patient out).

The trial duration for an individual patient is 12 months.

3.5 Premature Termination of the Trial

3.5.1 Termination of the Trial at a Single Site

The trial can be stopped/terminated at a single site if

- the protocol is not adhered to,
- the quality of data is deficient,
- there is inadequate recruitment.

The coordinating investigator decides whether or not to exclude the site, together with the sponsor and biometrician if appropriate.

Investigators and sites no longer participating in the trial must inform the coordinating investigator immediately and should provide justification for the decision.

Further treatment of patients still involved in the study is to be arranged together with the coordinating investigator.

3.5.2 Termination of the Whole Trial

The whole trial will be terminated prematurely if, in the opinion of the coordinating investigator or based on the DMC's recommendations, an unfavourable risk-benefit ratio develops or if continuation of the study no longer appears to be reasonably justified.

The trial can be terminated prematurely by the coordinating investigator in the event of

- serious adverse events /unacceptable toxicity
- changes in the risk-benefit considerations, e.g. as a result of unexpected adverse events
- proven superiority of the therapy arm (in the interim analysis)
- new insights from other trials
- an insufficient recruitment rate.

The final decision regarding the premature termination of the trial will be made by the authorized representative of the sponsor.

The approval can be rescinded or the study can be terminated by the responsible competent authority (Bundesinstitut für Arzneimittel und Medizinprodukte – BfArM) or the leading ethics committee, too.

In case, the study has to be terminated early (due to scientific, organisational, financial, or other reasons) the coordinating investigator will decide on probably meaningful data analyses after consultation of the responsible biometrician.

If about 2 thirds of the planned sample size were already treated and followed-up, selected descriptive analyses may be suitable. Priority and timing of those analyses will be done at the discretion of the ZKS Leipzig - KKS.

Neither a formal statistical analysis plan nor a comprehensive statistical report is mandantory in the situation of early termination although all efforts will be made to publish the meaningful results of trial.

4 TRIAL SUBJECTS

4.1 Inclusion Criteria

Patients must meet ALL of the following criteria:

1. **Acute-on-chronic liver failure (ACLF)** according to the consensus criteria recently defined by the CANONIC study group [Moreau 2013]. Patients with **acute decompensation** of cirrhosis [defined as acute development of one or more of the

following: ascites (onset and/or worsening), hepatic encephalopathy (onset and/or worsening), gastrointestinal haemorrhage, bacterial infection] are classified as ACLF if one of the following applies:

- single kidney failure (serum creatinine level ≥ 2 mg/dl) **or**
- single failure of one of the following organ systems: liver, coagulation, circulation, or respiration,

  together with

  a serum creatinine level ranging from 1.5 to < 2.0 mg/dl and/or mild to moderate hepatic encephalopathy **or**
- single cerebral failure together with serum creatinine level ranging from 1.5 to < 2.0 mg/dl **or**
- two or more organ failures.

Organ failures are defined according to the CLIF-C OFs [Jalan 2014].

2. Age ≥ 18 years, male or female
3. Written informed consent from patient, legal or authorized representative or a confirmation of justification of trial participation by an independent medical consultant

   PLEASE NOTE: In case of confirmation by the independent medical consultant a deferred informed consent from patient, legal or authorized representative has to be given.

4.2 Exclusion Criteria

Patients will be excluded for ANY ONE of the following reasons:

1. Prior not curatively treated or active malignancies
2. Sickle cell disease
3. Septic shock, defined by the following symptom complex: bacteraemia AND SIRS AND shock
4. WBC-count of **>** 50 x 10^9/L
5. Known HIV infection
6. Known intolerance to filgrastim
7. Suspected lack of compliance
8. Pregnant or nursing women
9. Fertile women (within two years of their last menstruation) without appropriate contraceptive measures[1] (implanon, injections, oral contraceptives, intrauterine devices, partner with vasectomy) while participating in the trial (participants using a hormone-based method have to be informed of possible effects from the trial medication on contraception).
10. Participation in other interventional trials.

Patients are excluded if they are at risk for suffering from major complications of G-CSF therapy such as crisis of sickle cell disease or potential stimulation of malignant cells due to

[1] In compliance with “Maintenance of the ICH Guideline on non-clinial safety studies for the conduct of human clinical trials for pharmaceuticals, M3(R1), CPMP/ICH/286/95, Note 3”

G-CSF in patients with prior malignancies. Patients with septic shock or HIV on top of liver cirrhosis are known to have a dismal prognosis and are unlikely to profit from G-CSF-treatment [Arabi 2012, Sherman 2013].

There are no reliable data about a critical spleen size that increases the risk for splenic rupture. Therefore patients with splenomegaly will not be excluded. But physicians should pay particular attention to clinical signs of splenic complications.

Thus, the eligibility criteria reflect the patient population, which may – according to the current knowledge – benefit from G-CSF treatment.

4.3 Justification for the Inclusion of vulnerable Populations

It is likely that the severity of the disease (ACLF) will result in a proportion of patients unable to provide informed consent before the beginning of the trial either in oral or written form, mainly due to cerebral failures.

The following describes the procedure for obtaining informed consent in patients unable to provide informed consent. General information on the Informed Consent Process is described in chapter 6.1.

In order to evaluate and respect the patient's will (**not to get an informed consent**), the trial staff will attempt to reach the patient's closest relatives and ask if any patient will is known and if an authorized or legal representative has been established. In addition to that, the trial staff will request information about the presumed patient will. The attempt of contacting the patient's relatives and the result of the contact are documented in the patient's file - with date and time.

If a living will with a DNR-Order (Do Not Resuscitate) exists or if it is presumed that the patient would have declined to participate in the trial, **then he/she is not to be included into the trial**.

If a legal representative or an authorized representative is in charge of the patient and the authorization also covers medical treatment, the legal representative or authorized representative then has to be contacted immediately and informed about the trial. The legal representative or authorized representative then decides whether or not the patient will participate.

If the closest relatives cannot be reached, the existence of a legal or authorized representative is unknown, there is no information about a living will at this timepoint and if it may be presumed that the patient would consent to participate, then the patient can be included in the trial via the regulations according to § 41 (1) AMG.

In these cases the following process should be followed:

An independent medical consultant examines the patient and confirms the patient's inability to provide consent as well as the urgency of participating in the trial with possible benefit to the patient. Note that the consultant is not permitted to be involved in the trial. The consultant must provide his decision regarding the justification in written form. Afterwards authorization of a legal representative must be requested from the responsible court as soon the patient has been included in the trial. This has to be documented in the patient's medical file. Subsequently, informed written consent must be obtained from the legal representative.

Patients initially unable to provide consent will be informed about the clinical trial as soon as they are able to do so and will then be asked to provide their written informed consent. This may also be documented in the patient file.

If a legal or authorized representative has given the informed consent and later on the patient refused to participate in the trial, then the stored data may continue to be used where necessary, in order to:

- Assess effects of the drug being tested
- Guarantee that the patient's personal interests are not adversely affected
- Comply with the requirements to provide complete documentation to obtain data

If the medical consultant confirms justification of trial participation and the patient or the legal representative thereafter does not give his informed consent, all stored blood samples will be destroyed, but the stored data may continue to be used as described above.

4.4 Participation in more than one Clinical Trial

During the verification of the inclusion and exclusion criteria the investigator/ his deputy or authorised medical staff of the study team checks if the patient is currently participating in any other interventional clinical trials covered by the AMG. Should this be the case, the patient will not be included. Moreover, by signing the informed consent form, the patient confirms that he/she is not participating in any other interventional clinical trial simultaneously.

4.5 Statement on the Inclusion of Dependent Individuals

During the screening procedure, all patients will be interviewed concerning any potential relationship to the investigator/ his deputy or to medical staff of the study team the coordinating investigator or the sponsor.

4.6 Rationale for Gender Distribution

We expect a gender ratio of 35:65 (female:male) [Moreau 2013]). All patients with acute-on-chronic liver failure (ACLF) that fulfil the inclusion and exclusion criteria will be informed about the clinical trial and asked to participate. Thus this trial may also allow the analysis of the incidence of ACLF depending on the gender of the patients

5 INVESTIGATIONAL PRODUCT

5.1 Trial Drugs

Commercially available, approved medication will be used. According to § 42 AMG and § 5 GCP-V, special labelling for the trial is not necessary. Nevertheless a minimal labelling of the commercially available, approved medication is carried out by the pharmacy of University of Leipzig.

A single syringe and the packages with syringes will provide the following information.

Labelling of single syringe:

Zur klinischen Prüfung bestimmt!
Prüfplancode: **GRAFT-Studie**
EudraCT-Nr. 2015-002212-32
Studienmedikation **Ratiograstim** ***30 Mio IE or 48 IE***
0,5 ml or 0,8 ml zur s.c.-Injektion

Pat.ID: I__I I__I I__I I__I – I__I

Labelling of outer package:

Zur Klinischen Prüfung bestimmt!

GRAFT-Studie
EudraCT: 2015-002212-32

Universität Leipzig

In accordance with § 5 paragraph 8 GCP-V, the trial samples may be provided together with accompanying documentation that permits the participants' safety, the traceability and identification of the medication and the clinical trial and that provides for the correct administration of the medication.

The summary of product characteristics (SmPC = Fachinformation) of trial drug is part of the appendix to the protocol.

Generic Name:	Filgrastim
Trade Names and manufacturer (if necessary):	Ratiograstim; ratiopharm GmbH
Drug allocation:	Drug will be shipped to trial site by the sponsor/pharmacy at the university Leipzig.
Formulation used:	syringe for subcutaneous injection
Packaging:	Packages with 1, 5 or 10 syringes (ready-to –use): - each 30 Mio IU (0.5 ml = 300 µg filgrastim) - each 48 Mio IU (0.8 ml = 400 µg filgrastim)
Storage conditions:	Store refrigerated at +2 to +8 °C, light protected
Stability:	Refer to the package insert
Preventive measures/Incompatibility:	see SmPC

5.2 Drug Accountability

The trial medication will not be shipped before all requirements for the start of the clinical trial have been fulfilled according to the internal SOP. The trial medication (**filgrastim**) will be shipped by the sponsor (executed by the pharmacy of the University of Leipzig) directly to the trial site. A detailed documentation of every shipment, number of syringes which will be used in this clinical trial will be made and kept on file. All shipments will be coordinated by ZKS Leipzig - KKS.

Medications have to be stored at trial site in a safe place between + 2°C and + 8°C according to manufacturers' storage conditions. The investigator is responsible for the appropriate storage at the study site and the detailed explanation of application of study drugs to patients.

After initiation of trial site by the sponsor (executed by the ZKS Leipzig – KKS) the delivery of study medication is triggered. The pharmacy of the University of Leipzig will send study medication to the site for first patients in the following manner:

<u>Packages for patients:</u>

for 4 patients: 48 syringes á 48 Mio IU (for a maximum of **12 injections per patient**) at least

for 2 patients: 24 syringes á 30 Mio IU (for a maximum of **12 injections per patient**) at least

Because of non-patient-specific pre-labelling of syringes they can be used for other patients at the trial site, in case one patient don´t use all 12 syringes.

Upon receipt of study medication the trial site will fax the medication receipt form to the sponsor. Trial sites will complete and update a site inventory log about all medication received at the site.

Storage of study medication will strictly follow of the manufacturer's storage recommendation. Temperature logs recorded from min-max thermometers will document the appropriate storage conditions for the respective study medication.

In a considerable number of cases, patients will be discharged during the 26-day G-CSF injection period. The administration of the remaining G-CSF injections should be preferably performed at the study sites (e.g. study ambulance). Self injections at home can be provided if adequate compliance can be presumed. These study subjects will be trained for self administration of subcutaneous injections and for a proper use of the syringes. The compliance should be evaluated at every patient visit. G-CSF in pre-filled syringes will be handed out and must be returned at the next study visit. Date and time of application should be recorded by patients in documentation sheet provided by the sponsor together with the syringes. The medical staff at the trial site and the monitor from the ZKS Leipzig – KKS appraise the drug accountability and thus the compliance of the patients as part of the on-site checks.

For each study subject an individualized drug accountability log will be recorded at trial site.

Unused study medication and empty syringes/packages are collected and destroyed at trial site after the monitor checked the drug accountability at trial site as part of the on-site verifications.

5.3 Administration of the Study Drugs

5.3.1 Dose, mode and scheme of intervention

Overall, **twelve doses of G-CSF** will be administered subcutaneously on **days 0-4,** then **every 3rd day over 26 days (days 7, 10, 13, 16, 19, 22, 25)**. This dosage scheme corresponds to that used by Garg et al. [Garg 2012] who could show a significant survival improvement indicating that this regime is effective in the clinical setting of acute-on-chronic liver failure.

Although a phase I trial in patients with compensated cirrhosis demonstrated a dose dependent effect on mobilization of CD34+ stem cells reaching its optimum with 10-15 µg/kg Filgrastim/G-CSF [Lorenzini 2008] other trials could show an effective mobilization in similar cohorts using 5 µg/kg [Di Campli 2007, Gaia 2006]. As the mobilization effect peaks not until day 4-7 after treatment initiation [Greenbaum 2011] an extension of the treatment period up to 26 days seems to be reasonable to prolong the positive effect on the hepatic repair capacity [Greenbaum 2011].

G-CSF will be injected subcutaneously after skin disinfection with pre-filled syringes (30 Mio IU or 48 Mio IU) on every pre-definded treatment day **at 12 am (with a window of one hour either way)**. If the injection coincides with a regular study visit study related drawing of blood samples as well as physical examinations should be performed within three hours prior to G-CSF administration. Referring to recommendations in SmPC for practical reasons and as part of the clinical routine G-CSF doses should be guided **by the body weight using a cut off value of 70 kg (≤ 70kg 30 Mio IU G-CSF, > 70 kg 48 Mio IU G-CSF).** If the patient has ascites requiring paracenteses the body weight measured immediately after the latest paracentesis should be used for dose calculation. If clinically indicated, paracentesis should be performed prior to treatment initiation. In all other cases

dose should be calculated using the baseline body weight. No further dose adjustment is required during the treatment episode.

If patients are discharged during the G-CSF treatment period pre-filled syringes will be handed out in a cool bag for transport. Patients will be trained for subcutaneous self administration and for a proper use of the syringes.

Definition of stopping rules

Stopping rules are defined (see section 6.6.1) according to risk considerations (see section 1.3) and known side effects of G-CSF.

In the case of a necessary interruption of G-CSF treatment due to a high value of WBC count the intervention period is **not to be extended over a total of 26 days including time of interruption**.

5.3.2 Compliance

In most cases study medication will be administered by medical staff, so that patients' compliance can easily be observed.

If patients are discharged during the G-CSF treatment period, compliance should be evaluated at every patient visit. The formerly handed out pre-filled syringes must be returned at the next study visit (see 5.2). If non-compliance is suspected the co-ordinating investigator or its deputy should be contacted in order to discuss study withdrawal.

5.3.3 Dealing with Side-effects

G-CSF (Filgrastim) is approved in Germany, but will be used in an experimental off-label manner during this trial.

G-CSF is routinely used for the treatment of neutropenia in different diseases and mobilization of hematopoietic stem cells in healthy donors for stem cell transplantation. The safety profile is well known. Side effects are very strongly dependent on the indication. Patients are excluded if there is a risk that they may suffer major complications of G-CSF therapy (for example: patients with sickle cell disease, malignant diseaes, septic shock, HIV).

Following the most important side effects are listed:

- splenomegaly, splenic rupture
- musculoskeletal pain
- headache
- capillary leak syndrome
- drug hypersensitivity
- cough, dyspnea, pulmonary edema, pulmonary hemorrhage
- interstitial lung disease
- nausea, vomiting, diarrhea
- tiredness
- baldness
- worsening of rheumatoid arthritis
- increased protein content in urine
- aortitis

For details of safety related aspects of filgrastim we refer to the SmPCs.

5.3.4 Alternative Medication

Not applicable

5.3.5 Contraindicated/Forbidden Concomitant Medication

There are no relevant interactions described with medication that are used in these patients.

In order to reduce severe complications/side effects patients with specific indications (see 0 exclusion criteria) and an associated increased risk of complications regarding G-CSF-intervention are not included in the study.

5.3.6 Overdose and Abuse

Overdose and abuse of medication will be dealt with like referred to in the SmPC of the drug.

6 INDIVIDUAL TRIAL PROCEDURES

6.1 Patient Information and Informed Consent

The investigator/deputy of investigator or authorized medical staff will explain to each trial subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort that may caused to each trial subject. Each trial subject will be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship to the treating physician. The patient will be provided with enough time to think about the participation in the study.

The informed consent will be given by means of a standard written statement, written in German in a non-technical language. The trial subject should read the statement and consider his/her decision before signing and dating the document, and should be given a copy of the signed document. No patient can enter the study before his/her Informed Consent has been obtained.

If a trial subject does not understand the document due to a language barrier only a certified translator must translate the document. The investigator must ensure and is responsible for the trial subjects' understanding of all aspects of the informed consent. In such cases the certified translator has to date and sign the informed consent, too.

In all other cases the trial subject cannot take part in the study.

The informed consent of the patient also refers specifically to the assessment and processing of data on the patients' health. The patient will be informed explicitly on the purpose and extent of the assessment and the use of his/her personal data, especially the health-related data.

6.1.1 Informed Consent in Patients not able to give Informed Consent by themselves

The details of the procedures for patients who are not able to provide informed consent in person are described in section 4.3.

Storage of blood samples for concomitant scientific projects (see chapter 9)

Patients will be asked to consent to a 10-years storage of blood samples for concomitant scientific projects including genetic analysis. These projects take place only at selected

centers. The selection of participating centers was performed in the context of pre study-visits together with investigator/deputy of investigator.

Consent can be given in a separate section of the general informed consent. If the patient does not give consent to the storage there is no blood sampling. The participation is optional and will not influence the main study.

6.1.2 Withdrawal of Informed Consent

Patients may withdraw their consent to participate at any time without giving reasons. Nevertheless, the patient should be asked for the reason of the premature termination after being informed that he/she does not need to do so. Information as to when and why a patient was registered/ randomized and when he/she withdrew consent must be retained in the documentation.

The patient is to be informed that in case of revocation of his/her consent, the stored data may be used further, as may be necessary to

- assess effects of the drug being tested,
- guarantee that the patient's personal interests are not adversely affected,
- comply with the requirement to provide complete authorisation documentation.

If patients refuse further drug administration but do not withdraw consent, patients remain in the study and data can be used for ITT-analysis.

6.2 Enrolment in the Trial

Generally, the local investigator in the trial site may prescreen subjects without obtaining written informed consent for general participation in the current study on the basis of pre-existing data (e.g., as available in medical records) or routine procedures that are necessary as part of the medical treatment of the patient **in respect of the presence of an acute-on-chronic liver failure** (see 4.1 Inclusion Criteria).

NOTE: Procedures performed solely for the clinical study can be performed only after obtaining informed consent according to the procedures described in chapters 4.3 and 6.1.

6.3 Baseline /Randomization (day -2 until 0)

Generally, subjects are enrolled to the trial by the local investigator at the trial site. After informed consent is given by the patient, general inclusion/exclusion criteria are checked as well as the demographic data.

In order to check all inclusion and exclusion criteria, several examinations including blood sampling/laboratory analyses during the patient's baseline investigation - **before randomization** - are necessary.

Baseline assessments

The baseline assessments:

- Assessment of ACLF grade using standardized method
- Assessment of concomitant diseases and medication

- Clinical assessment
    - Assessment of vital signs (arterial blood pressure, heart rate, temperature, respiratory rate, body weight - after the latest paracentesis in patients with ascites)
    - Blood gas analysis (capillary or arterial) **or** saturation of peripheral oxygen (PaO_2 **or** SpO_2) **and** fraction of inspired oxygen (FiO2)
    - ACLF Complications (Hepatorenal syndrome, variceal bleeding, ascites, hepatic encephalopathy)
- Local lab analysis
    - Liver function test including AST, ALT, gamma-GT, total bilirubin, albumin, alkaline phosphatase, INR,
    - Chemistry including sodium, potassium, urea
    - Creatinine
    - Hematology including erythrocytes, total leukocytes and differential count, platelets, haemoglobin, hematocrit
    - Urine analysis: urinary sodium excretion, urine status, urine sediment test
    - C reactive protein
    - Procalcitonin
    - Alpha fetoprotein
    - β-hCG test
- Further diagnostic procedures
    - Abdominal ultrasound - if not performed within **7 days** before enrollment
    - Duplex ultrasound of liver vessels for exclusion of a thrombosis (A. hepatica, Venea portae, Venae hepaticae) - if not performed within **7 days** before enrollment
- Optional: Blood sampling for scientific sub-projects at Baseline (see chapter 9)

As soon as all assessments required for the final check of eligibility are available, the patient will be randomized and the baseline assessments will be completed and recorded in the eCRF.

If the patient meets all inclusion criteria and none of the exclusion criteria, the randomization will be performed. The randomization form has to be sent as soon as possible to the ZKS Leipzig - KKS - data management by fax.

Fax: 0341 97 16 259

Fax randomization will be performed on working days from 8:00 to 18:00 clock. The trial sites will be informed by fax about the results of the randomization.

6.3.1 Discovery of a preexisting violation of the Eligibility Criteria after randomization

In general, the violation of eligibility criteria is not a reason for premature withdrawal of the patient from the trial therapy or from the whole trial.

If it is discovered that the patient was not eligible at the time of randomization, this has to be reported to the Data Management (ZKS Leipzig) as soon as possible. After discussing the

best decision for the individual patient and his/ her inclusion in the full analysis population between the investigator, the biometrician and project manager (e.g. via phone) the ZKS Leipzig informs the investigator /his deputy or authorised medical staff immediately as to what is to be done with the patient. The patient's data will further be recorded.

6.4 Description of Treatment Procedures

Immediately after randomization the patient will be treated in accordance with the allocated treatment arm: **standard care in combination with G-CSF injections** (experimental arm) or **standard care alone** (control arm).

6.4.1 G-CSF application

Overall, **twelve doses** of G-CSF will be administered subcutaneously – see 5.3.1:

- Day 0
- Day 1
- Day 2
- Day 3
- Day 4,
- subsequently every 3rd day over a period of 26 days as following:
  Day 7, 10, 13, 16, 19, 22, 25
  - independent of the regulär study visits (see Schedule of Assessments and Procedures)

The **first G-CSF injection** will be administered **not later than 48 hours** after the baseline blood sampling (local lab).

6.4.2 Standard treatment

Standard treatment has to be started/continued in both treatment groups, and should follow national guidelines, if not available international guidelines. This includes - if necessary – medical treatment with lactulose, L-ornithine-L-aspartate, albumin, vasopressors, antibiotics and N-acetylcysteine. If the precipitating event can be identified (e.g. infections, bleeding, drug toxicity, viral hepatitis), adequate treatment should be initiated. If indicated, patients might be supported by mechanical ventilation, vasopressors and renal replacement therapy.
Hypovolaemia should be treated by volume expansion.
Hepatitis B can be treated with nucleos(t)id analogues, if indicated.
Hepatitis C can be treated with interferon, ribavirin and/or direct-acting antiviral agents if indicated.
Hepatitis E can be treated with interferon or ribavirin, if indicated.
Alcoholic hepatitis can be treated with pentoxifylline and/or prednisolone, if indicated.
Liver transplantation can be considered, if necessary.
The use of liver support systems in this patient cohort is controversial and generally not indicated. Hence, its use should be avoided.

6.4.3 Treatment of subsequent ACLF episodes

After recovery from the first ACLF-episode, any subsequent ACLF-episode will be treated with standard medical care, irrespective of the randomization arm in order to evaluate the potential long-term consequences of G-CSF administration.

6.5 Description of Trial Visits

In both trial arms, regular visits are scheduled as follows:

Treatment phase

- Visit 1 [day 1 + 1 day]
- Visit 2 [day 4 +/- 1 day]
- Visit 3 [day 7 +/- 1 day]
- Visit 4 [day 14 +/- 2 days]
- Visit 5 [day 28 +/- 2 days]

Follow-up

- Visit 6 [day 90 +/- 7 days]
- Visit 7 [day 180 +/- 14 days]
- Visit 8 [day 360 +/- 14 days]

Generally, during **all** visits (V1 until V8), the following procedures will be performed:

- Assessment of adverse events and concomitant medication
- Assessment of ACLF complications (hepatorenal syndrome, variceal bleeding, ascites, hepatic encephalopathy)
- Assessment of vital signs (arterial blood pressure, heart rate, temperature, respiratory rate)
- Blood sampling (local lab analysis) for:
  - Liver function test including AST, ALT, gamma-GT, total bilirubin, albumin, alkaline phosphatase, INR
  - Chemistry including sodium, potassium, urea
  - Creatinine
  - Hematology including erythrocytes, total leukocytes and differential count, platelets, haemoglobin, hematocrit
  - C reactive protein

At specific visits following procedures will be performed:

- Taking blood samples (local lab analysis) for:

At visits V1 until V5:

- Procalcitonin

At visit V2:

- Alpha fetoprotein

- Clinical assessment:

At visits V3 until V5:

- Assessment of ACLF grade

- Blood gas analysis (capillary or arterial) **or** saturation of peripheral oxygen (PaO_2 or SpO_2) **and** fraction of inspired oxygen (FiO_2)

- Further diagnostic procedures:
  - Abdominal ultrasound – **at visits V4 until V8**
  - Duplex ultrasound (liver vessels) – **at visits V4 and V8**

At visits V1 until V8:

- Optional: Blood sampling for scientic sub-projects (see chapter 9)

6.6 Premature Termination of the Therapy or Follow-up

The date (as exactly known as possible) and if possible the circumstances/reasons for every premature termination of the therapy or follow-up will be recorded by the site where the patient was being treated and will be reported to the Data Management (ZKS Leipzig)

6.6.1 Premature Termination of the Experimental Therapy for Individual Patients

The experimental therapy may be terminated prematurely (see 5.3.1 Definition of stopping rules) because of the following reasons:

- G-CSF administration must be stopped if patients develop signs of filgrastim related anaphylactic reaction, splenic rupture, pregnancy or if any other severe and threatening intolerance reaction is suspected.
- According to the SmPC G-CSF administration should be discontinued if WBC count raises to > 70 x 10^9/L but can be continued again if WBC count recedes 50 x 10^9/L.

  In the case of a necessary interruption of G-CSF treatment due to a high value of WBC count the intervention period is not extended over 26 days.
- in case of side effects that in the discretion of the investigator and /or the coordinating investigator are indicating the termination of standard treatment,
- at the judgment of the investigator for reasons of medical prudence, or
- on request of the patient.

Premature termination should be avoided. In case the patient misses the scheduled visits, the investigator may contact the patient directly, in order to motivate him/her for further continuation.

In case of premature termination of treatment it is necessary to document the reason of termination and the current condition of the patient.

All further study visits until day 360 will take place as planned and described above. Termination of experimental therapy does not mean that the patient is off-study.

If the patient is discharged prior to treatment completion G-CSF administrations should be continued.

6.6.2 Premature Termination of the Follow-up for Individual Patients

All randomized patients will be followed up until day 360. Premature termination of G-CSF therapy in the experimental arm does not lead to individual study termination.

The only circumstances in which a premature study termination (i.e. no further study visits) in a randomized patient is unavoidable are:

- withdrawal of informed consent,
- complete loss of contact to the patient or
- death of the patient.

Each premature termination of the trial has to be documented by the responsible investigator. If possible date, circumstances of, reason for the termination, and - if applicable - the final status of patient regarding death and OLT should be documented and communicated to the Data Management (ZKS Leipzig).

6.7 Plan for Further Treatment

As described in section 6.4.3, after recovery from the first ACLF-episode which led to inclusion into the trial, patients will be treated with standard medical care at the discretion of the treating physicians – in both treatment arms.

After the last study visit (V8) all patients will be treated according to the current treatment guidelines.

7 ADVERSE EVENTS (AE/SAE)

7.1 Adverse Events (AE)

7.1.1 Definition

An Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation subject (in whom a pharmaceutical product was administered) which does not necessarily have to have a causal relationship with this treatment (ICH-Guideline E2A).

Adverse Events encompass any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease that arise newly or worsen after the inclusion of the patient into the trial.

7.1.2 Documentation and Reporting

Adverse events (AE) will be documented for patients in both treatment arms (experimental and control arm) at every visit **from Day 0** (in experimental arm: starting with the **first injection of G-CSF**) until visit **V5** (= 28 days).

Exceptions are newly developed malignant diseases. These have to be documented up to the end of the study (= day 360) as AE.

The following events could be ACLF-related clinical outcomes/endpoints. Occurrence will be documented at each visit, details will be documented within the CRF in addition and independently from the AE-form:

- Complications of ACLF (hepatorenal syndrome (HRS), variceal bleeding, ascites (onset and/or worsening), hepatic encephalopathy (HE) (onset and/or worsening)
- Infections (proven infection necessitating systemic use of antibiotics); i.e.: spontaneous bacterial peritonitis, pneumonia, urinary tract infection, infections associated with medical interventions (catheters), sepsis of unkown origin, cellulitis, and others
- Relevant changes of liver function during the course of treatment and follow-up

AE reports comprise any newly reported events as well as worsening of pre-existing conditions (i.e. increased intensity/grading) during the predefined reporting period of events.

An assessment of laboratory values regarding clinical relevance is carried out by authorized medical staff. Only in the case of clinically significant alterations of lab values an AE report is required.

In addition at specified time points (see Schedule of Assessments and Procedures) physical examinations will be performed with significant pathologic findings being reported as adverse events.

AEs are documented on specified AE forms provided in the CRF. Documentation includes the type of AE (symptom, sign or/and diagnosis), start and end dates, measures taken regarding G-CSF application, outcome, assessed causal relationship of the AE with the G-CSF treatment (see also 18.1).

G-CSF increases the amount of circulating bone marrow derived stem cells and WBC. That means that an increased WBC count in patients of the experimental arm (G-CSF) does not necessarily leads to an AE. But as soon as WBC counts **>70 x 10^9/L** are measured, an AE report is required. This is in accordance with the information in the SmPC, that G-CSF administration should be discontinued, if WBC count raises to >70 x 10^9/L.

In the control arm the clinical relevance of an increased WBC count and an associated AE report remains at the discretion of the authorized medical staff.

Furthermore the following exceptions are defined in case of an orthotopic liver transplantation (OLT) – see 7.4.1:

- If an OLT is occured before day 28, AEs have to documented **up to 3 days** after the last injection of G-CSF in the experimental arm,
- OLT itself must **not** be reported as AE; a special CRF page is to be filled in those cases

For adverse events that occur in the context of the OLT, the following reporting requirements apply:

- no relationship with G-CSF, but with the OLT -> **no** AE reporting necessary
- relationship with G-CSF possible -> AE reporting necessary
- relationship with G-CSF or OLT inconclusive -> AE reporting necessary

7.2 Safety Analysis

At every visit all patients will be monitored closely by clinical assessment for safety relevant issues:

- vital signs
- laboratory findings

7.3 Concomitant Diseases

At baseline all ongoing or relevant past diseases or medical conditions are recorded. A change of intensity of a pre-existing condition during the trial will be reported as an adverse event.

7.4 Serious Adverse Events (SAE)

7.4.1 Definition

An Adverse Event is defined to be serious according to ICH-Guideline E2A, paragraph IIB, if it

- **results in death**,
- **is life-threatening**,

  Note: The term “life-threatening” in the definition of “serious” refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death had it been more severe.

- **requires in-patient hospitalization or prolongation of existing hospitalization**,
- **results in persistent or significant disability/incapacity or**
- **is a congenital anormal/birth defect**.

Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

The SAE reporting period is defined from Day 0 (in experimental arm: starting with the **first injection of G-CSF**) to V5 (Day 28) **except** for newly developed malignancies, as it is defined for AE in section 7.1.2.

Furthermore the following exceptions are defined in case of an orthotopic liver transplantation (OLT) - see 7.1.2:

- If an OLT is occured before day 28, SAEs have to documented **up to 3 days** after the last injection of G-CSF in the experimental arm,
- OLT itself must **not** be reported as SAE; a special CRF page is to be filled in those cases

For serious adverse events that occur in the context of the OLT, the following reporting requirements apply:

- no relationship with G-CSF, but with the OLT -> **no** SAE reporting necessary
- relationship with G-CSF possible -> SAE reporting necessary
- relationship with G-CSF or OLT inconclusive -> SAE reporting necessary

Further exception:

Any diagnostically confirmed aortitis causes a SAE reporting even if it does not meet the SAE criteria.

7.4.2 Documentation and Reporting Obligations: INVESTIGATOR

Serious Adverse Events have to be documented on the SAE-forms and must be reported by the investigator, his deputy or authorised medical staff to the sponsor **immediately**. If more information about the SAE becomes available later, it must also be reported to the sponsor immediately.

Serious adverse events will be documented at every visit from baseline until visit V5 (= 28 days) for all patients (see above-mentioned expections in case of an OLT and newly developed malignancies).

In the event of a patient's death, the investigator/ the deputy or the authorised medical staff provides the leading ethics committee, all involved ethics committees, the competent authoritiy and the sponsor with all further information needed to fulfil their tasks **upon request**.

In all the reports, personal data have to be pseudonymized by using the patient's identification code and blackened if applicable. It must be possible to relate the initial and all follow-up reports to each other by means of the patient identification number and a concurrent SAE number.

The investigator, his deputy or the authorised medical staff must report every Serious Adverse Event immediately after becoming aware of the event to the following address:

ZKS Leipzig - KKS/ Arzneimittelsicherheit
Universität Leipzig
Zentrum für Klinische Studien Leipzig – KKS
Härtelstr. 16-18, 04107 Leipzig
Telefon: +49/341/97-16129
Fax: +49/341/97-16278
E-mail: pharmacovigilance@zks.uni-leipzig.de

7.4.3 Documentation and Reporting Obligations: SPONSOR

After the KKS receives the SAE, it is immediately passed on to the coordinating investigator for the medical assessment.

In the KKS the SAE data are entered into the SAE database immediately and the MedDRA coding takes place simultaneously.

Then forwarding as per law and as described in Chapter 8.5 only for Suspected Unexpected Serious Adverse Drug Reactions (SUSARs) takes place if applicable.

Details of the sponsor's documentation and reporting obligations will be specified in a trial-specific pharmacovigilance plan, which will be written and finalised alongside with this protocol.

7.5 Periodic Reports

7.5.1 Annual Safety Report

The sponsor has the duty to submit a safety report annually or upon request (Annual Safety Report, ASR[2]). This report with documentation of relevant safety issues will be sent to the leading ethics committee and the competent authority.

The key date is the date of the first authorization of the clinical trial by the competent authoritiy. All data obtained up to this date (each year) will be included in the ASR. Beginning with the key date, there is a time-limit of 60 days for the preparation and submission of the ASR.

The ASR is written by the coordinating investigator in cooperation with the project manager at the KKS and the responsible biometrician.

[2] See "Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use").

7.6 Suspected Unexpected Serious Adverse Reactions (SUSAR)

7.6.1 Definition

Suspected Unexpected Serious Adverse Drug Reactions (SUSARs) are side-effects (probably or definitely connected with the administration of the investigational product), the nature or severity of which are inconsistent with the information available about the product. Reference/Information about the trial product is contained in the SmPC (Summary of medicinal Product Characteristics).

7.6.2 Documentation und Reporting Obligations

Information *for SPONSOR*

The sponsor submits all information available about a SUSAR immediately to the leading ethics committee, the competent authority, and to all participating investigators, at the latest within 15 calendar days after the event becomes known to the sponsor.

For every SUSAR that results in death or a life-threatening condition, the leading ethics committee, the competent authority, and all participating investigators must be informed by the sponsor within 7 calendar days after the event becomes known to the sponsor. Additional information has to be given within 8 further calendar days.

Details of the sponsor's documentation and reporting obligations will be specified in a trial-specific pharmacovigilance plan which will be written and finalised alongside with this protocol.

Information for INVESTIGATOR

The investigator passes down all relevant information concerning the SUSAR to all participating medical staff at his/her trial centre. This has to be confirmed by the investigator by signing an acknowledgement document.

7.7 Other Safety Relevant Issues

Other safety issues also qualify for expedited reporting where they might influence the current benefit-risk assessment of an investigational medicinal product or would be sufficient to consider changes in the investigational medicinal products administration or in the overall conduct of the trial, for instance:

New events related to the conduct of a trial or the development of an IMP likely to affect the safety of subjects, such as:

- a serious adverse event which could be associated with the trial procedures and which could modify the conduct of the trial,
- a significant hazard to the subject population such as lack of efficacy of an IMP used for the treatment of a life-threatening disease,
- a major safety finding from a newly completed animal study (such as carcinogenicity),
- a temporary halt of a trial for safety reasons if the trial is conducted with the same investigational medicinal products in another country by the same sponsor
- recommendations of the DMC, if any, where relevant for the safety of subjects.

The sponsor, together with the DMC (see 12.5), if appropriate, decides if the number of events or qualitative changes in the expected SARs comprise a safety issue and must be reported.

7.8 Therapeutic Procedures

If a patient requires treatment as a result of an Adverse Event, then it must meet the recognized standards of medical care in order to restore the patient's health. Appropriate resuscitation devices and medication must be available in order to treat the patient as quickly as possible in the event of an emergency.

The action taken to treat the AE/SAE must be documented by the investigator or authorized designee either in the appropriate CRF and/or using additional documents.

7.9 Dealing with Pregnancy

Every pregnancy that occurs while taking part in the trial must be reported to the ZKS Leipzig - KKS by the investigator/ the deputy or the authorised medical staff within 24 hours of having learned of it.

ZKS Leipzig /Pharmacovigilance
Universität Leipzig
Zentrum für Klinische Studien Leipzig – KKS
Härtelstr. 16-18, 04107 Leipzig
Telefon: +49/341/97-16129
Fax: +49/341/97-16278
E-mail: pharmacovigilance@zks.uni-leipzig.de

Pregnancies have to be reported by using the form „Report on the arising of a pregnancy during exposition to a trial medication". Severe side effects and complications during a pregnancy as well as congenital birth defects are Serious Adverse Events per definition and therefore have to be reported additionally on the Serious Adverse Event form according to the reporting procedures described above.

The outcome of a pregnancy has to be reported on the form "Report on the outcome of a pregnancy during/after exposition to a trial medication". This form will document the outcome of the pregnancy, including a spontaneous or voluntary abortion, details of the birth process, the presence or absence of congenital malformations and birth defects, maternal or foetal complications and the potential relationship to the trial drug.

Collecting data concerning the outcome of a pregnancy is only permitted if the trial subject puts down her permission in writing beforehand.

The pregnancy of a patient should not automatically lead to premature termination of the study. The attending physician must decide together with the coordinating investigator on the continuation or termination of the study.

8 BIOMETRY

8.1 Biometrical Aspects of the Trial Design

This is a randomized parallel-group trial with two groups:

- experimental group, in which patients receive 12 doses of G-CSF in addition to standard care over 26 days after randomization
- control group, in which patients receive standard care as per current treatment guidelines only.

All patients will be followed for 360 days after randomization.

8.1.1 Measures to Prevent Bias

Randomization

Randomization will be performed centrally via a secure web-based tool. Allocation to the treatment arm (randomization ratio 1:1) uses randomized blocks. The algorithm will balance the number of patients in each treatment arm according to centre. According to Moreau et al [[Moreau 2013] grades of ACLF present a major predictor of mortality and therefore the baseline ACLF score was originally considered as stratification criterion in the planning stage of the trial to account for prognostic heterogeneity.

However, it became apparent that most of the trial centres were unfamiliar with its scoring scheme so that we decided to document all subcriteria essential to calculate it but not to use it as stratum in the first months of the trial. Together with ASR analyses the distribution of ACLF grades within the study population will be evaluated to decide whether it might be necessary to adjust for ACLF severity between the treatment arms and to ensure the rate of events as expected according the the calculation of sample size.

The minimisation algorithm to be used will incorporate a degree of random allocation, in order to avoid potential loss of allocation concealment within centres.

Blinding

Placebo control is neither feasible nor ethically justifiable in our study setting. Blinded treatment may lead to misinterpretation of a G-CSF associated white blood cell count (WBC) increase being a consequence of an infectious complication. In addition, G-CSF is often associated with non-serious side effects like bone pain and irritation of the injection site which also hampers blinding of the treatment arm. As an increase in WBC may not be used as marker of infection in G-CSF-treated patients, SIRS-Criteria (except for leukocytosis), C-reactive protein (CRP), procalcitonin (PCT)-levels and if required culture based microbiological diagnostics will be used as standard screening tools for infectious complications throughout the trial period (for both treatment groups). Delayed diagnosis of infectious complications is not expected, as CRP and procalcitonin-levels are reliable markers for infections in liver cirrhosis [Papp 2012; Lazzarotto 2013].

8.2 End Points

8.2.1 Primary End Point

Transplant-free survival at 90 days, defined as time from randomization to death or OLT, censoring patients alive without OLT at the date of last information.

8.2.2 Secondary End Points

Secondary endpoints are

- **Overall survival** at 360 days, defined as time from randomization to death, censoring patients alive at the date of last information (independent from an OLT done or not).
- Transplant-free survival at 360 days
- **Complications of ACLF** within 90 days/within 360 days. For each patient, every complication will be recorded including onset, HRS type 1/2, classification of ascites, and/or HE grade.

- **Infections** within 90 days/within 360 days necessitating systemic use of antibiotics

  Complication (by highest scoring if applicable)/infections will be analysed per period of analysis regarding occurrence and number of episodes (if episodes delimitable)

- Liver function during the course of treatment and follow-up.

  *The **MELD-Score** and the **Child-Pugh-Score** will be derived within the data analysis at every visit based on the detailed information from the DB.*

8.2.3 Further characteristics for evaluation

Further characteristics of interest are (e.g.):

- frequencies (per group) of organ failures contributing to ACLF during the course of treatment and follow-up, i.e. of
  - renal function,
  - cerebral function,
  - coagulation,
  - respiratory function,
  - cardiovascular function.

  Organ failures will be assessed according to the CLIF-C OFs conventions [Jalan 2014]. Respective laboratory values and further necessary items will be monitored during the course of treatment and follow-up.

- **Duration of initial hospital stay**. Time to discharge will be defined as time from randomization to day of discharge, censoring patients deceased during the initial hospital stay.

Assessment of safety:

In addition to the complications of ACLF listed above, further AEs and SAEs will be assessed.

8.3 Statistical hypotheses

It is to be assessed whether there is a difference in the transplant-free survival up to 90 days after randomization (visit 7) between the two study groups. The statistical hypotheses are:

- H_0: Transplant-free survival (experimental group) = Transplant-free survival (control group)
- H_A: Transplant-free survival (experimental group) ≠ Transplant-free survival loss (cont$_r$ol group)

8.4 Sample Size Discussion

8.4.1 Estimation of effect size

Based on the CANONIC Study, we expect a transplant-free survival rate (as defined in 8.2.1) of 42 % at day 90 in the control group. The two small randomized trials investigating G-CSF in ACLF both show a significant beneficial effect of G-CSF: Garg et al [Garg 2012] report survival rates at 60 days of 66 % vs. 26 % in n=47 patients, Duan et al [Duan 2013] 48 % vs. 21 % at 3 months in n=55 patients. However, both trials have considerably lower survival rates with standard treatment than expected in our setting. This may have contributed to the

magnitude of the effect. Thus, we aim to detect an absolute difference of 20 % (from 42 % to 62 %, hazard ratio 1.815) in transplant-survival at day 90. This is still a clinically highly relevant difference, but accounts for possible overestimation in the two pilot trials.

8.4.2 Statistical error probabilities

The study is designed to ensure a type-I error rate of $\alpha \leq 0.05$ and a power $1-\beta$ of 0.9.

8.4.3 Drop-outs

Due to the short observation period for the primary endpoint and to the serious medical condition of the patient population, we expect a drop-out rate (i.e. patients with missing information on transplant-free survival for the complete observation period of 90 days) not exceeding 10 %. This is in line with drop-out rates reported so far [e.g. Kribben 2012, Garg 2012]. Since the primary endpoint is a time to event variable, all patients will be included in the primary analysis with their individual observation time.

8.4.4 Sample size calculation

With these assumptions, a total of 262 patients have to be analysed to achieve a power of 90 % at a significance level of 5 % using a two-sided log-rank test (NQuery Advisor ® 7.0).

Taking a drop-out rate of 10 % into account, 292 patients will be randomized.

8.5 Statistical Methods

8.5.1 Analysis Population

Full analysis set

The full analysis set (FAS, also called intention-to-treat (ITT) population) is defined by all randomized patients with valid informed consent.

Per protocol set

The per-protocol (PPS) set is defined by all patients belonging to the ITT without major violations of the study protocol.

The following protocol violations are classified as major:

- Violation of an eligibility criterion;
- Patients who did receive less than 75 % of the intended total dose of G-CSF or who have received G-CSF on less than 9 treatment days for *reasons others than early death, early OLT, or (serious) adverse events or relevant clinical reasons*;

This is not an exclusive list. In the light of protocol violations which actually occur during study conduct, all major protocol violations will be defined, e.g. as part of the statistical analysis plan. The completed list will be finalised before database closure and start of the final analysis.

Safety analysis set

The safety population is defined by all randomized patients belonging to the FAS. In safety analyses, patients will be classified whether or not they received at least a single G-CSF injection, irrespective of the randomized group allocation.

8.5.2 Planned Methods for Analysis

The **primary efficacy analysis** of a treatment effect on transplant-free survival within 90 days will be performed by Cox regression adjusting for ACLF grade to gain power. Previous data suggest that the proportional hazard assumption is justified [Garg 2012, Duan 2013]. Confirmatory analysis follows the intention to treat principle and will be based on the full analysis set (FAS). Hazard ratios for treatment effect and ACLF grade and their confidence intervals will be presented.

In addition, a per protocol analysis will be performed for sensitivity reasons, excluding all patients with major protocol violations (e.g. non-compliance with randomized treatment, violation of eligibility criteria).

Additional explorative multivariate analyses will be performed to assess the prognostic value of further relevant baseline parameters.

Secondary endpoints: Time to event endpoints will be described by Kaplan-Meier curves and compared with the log-rank-test. Rates of complications and infections will be compared by chi-square test. Odds ratios with 95 % confidence intervals will be provided. Time courses of liver scores (MELD; Child Pugh), grade of ACLF and lab values will be described. Due to the explorative nature of these analyses, missing data will not be imputed.

No confirmatory subgroup analyses are planned. Exploratory subgroup analyses will focus on the type of underlying liver disease (alcoholic hepatitis, viral hepatitis).

Safety issues will be carefully monitored. Annual safety analyses will be performed and presented to the DSMB.

Details of statistical analyses will be pre-specified in a statistical analysis plan according to ZKSL SOPs. The trial will be reported according to CONSORT criteria.

8.6 Statistical Monitoring

The trial conduct will be regularly supervised by means of central statistical monitoring to ensure data quality. The objectives are

- to detect safety relevant signals as soon as possible
- to detect non-compliance and relevant protocol violations and to prevent their future occurrence by prompt reaction

Central statistical monitoring will start 9 months after inclusion of the first patient before the analyses for 1st annual safety. Findings will be queried for clarification with the centres. Non-compliance and/or PVs will be discussed at ZKS Leipzig study team, with the principle investigator and with the respective centre(s).

8.7 Interim Analysis

One interim analysis is scheduled after outcome of 50 % of the patients has been documented, allowing to detect marked superiority of the experimental treatment. Multiplicity adjustment follows O'Brien [1979] with nominal significance levels p=0.005 at interim analysis and p=0.048 at final analysis. The interim analysis is designed to have a power of at least 80 % to detect a difference in transplant-free survival in the order of 33 % or higher. Results of the interim analysis will be presented to the Data Monitoring Committee (DMC). Only one interim analysis is planned for the following reasons: an interim analysis with less than 50 % of the patients would not have adequate power to detect even marked differences. On the other hand, since we expect a recruitment period of 24 months and since the primary outcome is assessed 90 days after randomization, for logistical reasons it seems not realistic to have the results of a second interim analysis before end of recruitment.

8.8 Final Analysis

Final analysis will be performed when the data of all enrolled patients have been collected, all DM procedures have been finalized and the data base has been closed.

9 CONCOMITANT SCIENTIFIC PROJECTS

Participation in concomitant scientific projects is optional but highly encouraged in order to obtain valid information about pathophysiological processes in acute-on-chronic liver failure as well as underlying mechanism by which G-CSF improves patients' outcome. The selection of participating centers was performed in the context of pre study-visits together with investigator/deputy of investigator. Side projects must not interfere with the primary study objectives. Patients can be addressed at selected trial sites in order to participate in additional projects.

Note!

To reduce the risk for patients, a critical hemoglobin level of 4.0 mmol/L (6.44 g/dl) was determined. Once this safety limit is not reached blood samples for subprojects must not be taken.

If patient consent to the participation the blood samples will be stored at the study site and sent to the sponsor for potential future analysis (projects A and B). In case of participation in project C blood sampels for project A and B can be send alternatively to the Experimental Surgery laboratory at the Charite Berlin. Handling of samples for project C will be described at this section.

A) Storage of blood samples for scientific projects

On the basis of the these blood samples specific questions concerning the value of novel biomarkers, that include but are not limited to bacterial and fungal DNA, cytokines, inflammatory markers and the isolation of PBMC and their microparticles in correlation with the clinical course. Treatment success and rate of complications in relation to the study treatment are explored. EDTA- and serum blood (5.7 ml) will be collected prior to treatment (baseline) and at each study visit (V1-V8) after start of treatment.

B) Genetic analysis of a blood sample

In this subproject it will be investigated whether the individual genomic structure differently influences the rate of complications. Specific receptors on immune cells that play an important role in the elimination of bacteria are known to significantly impact the clinical course and risk of infections in patients with liver cirrhosis and acute-on-chronic liver failure if single nucleotide polymorphisms (SNP) occur. We therefore expect an association between immune cell SNPs and patients' outcome within the study. EDTA-blood (2.7 ml) will be collected at baseline **or** at a later visit.

These investigations **(A + B)** are carried out in the laboratory and on behalf of the study sponsor, represented by the coordinating investigator.

Blood samples will be collected during routinely performed study visits. After preparation the samples will be stored at -80 °C at the study site and sent in bulk to the laboratory of the sponsor, represented by the coordinating investigator. If patients consent to project C blood samples for project A and B can be send alternatively to the Experimental Surgery laboratory at the Charite Berlin. In this case sample preparation is not mandatory.

C) Bone marrow responses to G-CSF and modulated immune functions in patients with ACLF

These investigations are carried out at the Experimental Surgery laboratory, Department of General-, Visceral- and Transplant Surgery, Charité-Universitätsmedizin Berlin, Campus

Virchow (PD Dr. Moritz Schmelzle) on behalf of the study sponsor.

EDTA-blood (10 ml each) will be collected at all collaborating centers prior to treatment (baseline, Day 0) and 1d, 4d, 7d, 14d, 28d, 90d d after start of treatment. Age and sex matched liver healthy patients scheduled for minor operations, e.g. hernia repair, will serve as controls. Results will be analyzed in particular with regard to:

a) differences between patients in treatment arms A and B,

b) differences in cellular responses to G-CSF normalized to pre-treatment levels and

c) differences between survivors and non-survivors (defined as dead or transplant).

Full blood will be shipped within 24h at 4°C (cool packs) and samples will be processed and analyzed standardized within further 24h. Extensive tests have been carried out in the laboratory, which confirmed cellular stability and preservation of relevant surface markers under experimental conditions. Parts of the blood will be analyzed immediately after arrival. For that, red blood cells will be lyzed, samples will be washed, stained and surface markers will be analyzed by flow cytometry. In concert with the description of cellular responses (mobilization and function of immune cells and hematopoietic stem cells (HSC) and immune-cell mobilization), detection of cytokines and growth factors might further help to understand complex interactions involved in G-CSF dependent attenuation of liver injury. Other parts of the blood will be centrifuged and plasma will be stored at -80°C upon further analysis. Levels of different cytokines and growth factors will be subsequently determined utilizing enzyme-linked immunosorbent assays (ELISA). Levels of endogenous cytokines, e.g. G-CSF, might indeed help to select ACLF patients for treatment with G-CSF. Data will be correlated with routinely assessed clinical parameters.

Additional to descriptive analysis, we plan to elucidate the effect of G-CSF on functional properties of human stem cells (HSC) and neutrophils. Heparinized blood samples (10 mL) that have been collected at baseline and at day 4 visit (V2) after start of treatment will be shipped within 24 hours unrefrigerated and immediately processed after arrival for functional tests, in vitro.

10 ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

10.1 GCP-Statement

All persons participating in the trial (sponsor, authorized representative of the sponsor, investigators, etc.) commit themselves to observe the Declaration of Helsinki (Version Sommerset West 1996), as well as all pertinent national laws and the ICH guidelines for Good Clinical Practice (GCP) issued in June 1996 and CPMP/ICH/135/95 from September 1997.

10.2 Initial Submission

10.2.1 Submission to the Ethics Committee and Competent Authority

Prior to submitting the trial related documents to the leading (and involved) ethics committee(s) and the competent authority, the sponsor must enter the trial into the European database for clinical trials (EudraCT).

Afterwards, the protocol and all other associated documents according to GCP-V § 7 will be submitted to the leading ethics committee for approval. Parallel to the submission to the leading ethics committee (EC), each participating EC is informed of the submission and also receives a copy of the documents including those of the trial sites, which they have to

approve. At the same time the study documents will be submitted to the competent authority (BfArM) according to the requirements of GCP-V § 7.

The trial can start only after obtaining a positive review by the leading ethics committee and approval from the competent authority. The written approval of the EC must be filed in the trial master file (TMF). Additionally, every participating centre must receive a copy of these documents to be filed in the investigator site file (ISF).

10.3 Protocol Amendments

Changes made to the protocol that was appraised positively by the ethics committee and approved by the competent authority must be positively reappraised and approved if the changes

- are such that they may affect the subjects' safety, e.g. fundamental changes to the diagnostic and therapeutic procedures (i.e. volume of blood samples for different laboratory analyses)
- result in further data collection that necessitates changes to the patient information and/or informed consent form,
- affect the interpretation of the scientific documents upon which the trial is based or the significance of the results of the trial,
- significantly affect the leadership or conduct of the trial, or
- concern the quality or the innocuousness of the investigational drug.

In order to ensure most comparable conditions during trial conduct and in the interest of valid statistical analyses, the investigators, the coordinating investigator or any other person involved in the trial conduct may not alter the study conditions agreed upon and set out in this protocol.

Amendments should be made only in exceptional cases. Any amendment must be set out in writing, at the same time giving the reasons, and signed by all parties concerned. The amendment then becomes part of the study protocol, and is to be filed in the Trial Master File (TMF).

Amendments which might have an impact on the well-being of the subject (major amendments) such as the use of additional invasive procedures require an additional approval by the Ethics Committee (EC) and by the competent authority. In addition, a further informed consent form is to be signed by all trial subjects enrolled in the trial who might be affected by the amendment. In case of substantial changes new approvals of the leading ethics committee and approval of the competent authority are required before the changes become effective. Minor changes will only be submitted to the Ethics Committee and the competent authority in a written form.

The investigator may implement a deviation from, or a change of the protocol to eliminate an immediate hazard(s) to trial subjects without prior EC approval opinion. As soon as possible, the implemented deviation or change, the reason for it, and if appropriate, the proposed protocol amendment(s) should be submitted to the coordinating investigator for agreement.

11 DOCUMENTATION

11.1 Case Report Forms (CRF)

The Case report Form (CRF) will be designed by the ZKS Leipzig – KKS in cooperation with the Coordinating Investigator and provided as electronic form (eCRF).

The Investigator or a member of the study team authorised for this task will connect to the database via internet and enter data directly into the database via eCRF data entry masks.

In order to facilitate the documentation as per protocol in case of malfunction of the electronic system or any of its components, a paper version of the CRF will be additionally provided. The content of this paper version has to be transferred to the eCRF as soon as the electronic system is available again.

The eCRF has to be filled in shortly after each study visit.

Each eCRF page has to be signed electronically by the investigator or a therefore authorised member of the study team by assigning the status “completed”. This represents the electronic equivalent of a signature on paper and confirms that all data on the eCRF is correct and hasn’t been changed. If a value gets changed on the eCRF later on, the status “completed” will be set back automatically and has to be assigned again by the investigator or an authorised member of the study team. This ensures that changes on the eCRF will be dated and signed as well. All entries and data changes will be tracked automatically including date, time and person who entered/changed information (audit trail). Major correction or major missing data have to be explained.

If the Investigator authorises other members of the study team to enter and sign CRF data, their name, initials, position, signature must be supplied to the Sponsor or its authorised representative via Staff Signature und Delegation Log.

However, **the investigator has final responsibility** at all times for the accuracy and authenticity of all clinical and laboratory data entered in the eCRF.

An eCRF will be provided for each patient. The patient will be identified as per the Patient-ID only. All information required by the protocol and therefore collected during the clinical trial must be recorded by the Investigator or an authorised member of the study team as source data in the source documentation for the study.

Source data according to ICH-GCP E6 are defined as any information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

For each trial site, it will be defined where the respective source data for specific CRF entries are filed (Source Data Agreement).

11.2 Data Management

The EDC Tool eData Entry (eDE) by OmniCom will be used for creation of the study database. The database will be validated according to the Standard Operating Procedures (SOPs) of the ZKS Leipzig - KKS prior to data capture.

The information entered into the eCRF by the investigator or an authorised member of the study team is systematically checked for completeness, consistency and plausibility by routines implemented in eDE, running every night. Error messages generated by these routines will be checked by data management staff of the ZKS Leipzig - KKS.

Discrepancies, errors or omissions will be passed to the investigator or an authorised member of the study team at the investigational site via query management tool of eDE. The investigator will receive notification of all queries concerning his/her investigational site. The ZKS Leipzig - KKS will supervise and support the solution of queries. Corrected data will be re-checked by automatic routines during the night after entry. In case a query cannot be solved, the Data Management staff of the ZKS Leipzig - KKS may close the query. This shall happen in agreement with the study biometrician and clarification, if the information addressed by the query is relevant for the results of the study, or not.

During the whole course of the study, a backup of all data is made on a daily basis. Unauthorised access to patient data is prevented by the access concept of the study

database which is based on a strict hierarchy and role model. Any change of data (e.g. when data is changed in the database during query management) is recorded automatically via audit trail within the database.

At the end of the study, once the database has been declared complete and accurate, the database will be locked. Thereafter, any changes to the database are possible only by joint written agreement between coordinating investigator, biometrician and data manager.

11.3 Archiving

All relevant trial documentation (Trial Master File), the electronically stored data, the original CRFs and the final report will be stored for at least 10 years at the KKS after the trial's completion.

At the investigating sites, the investigators' files, patient identification lists, signed written consent forms, copies of all CRFs and the patients' files will be stored for at least 10 years after the trial's completion. If local rules or other legal requirements require longer periods of archiving, then these are to be met.

12 SUPERVISION OF THE CLINICAL TRIAL

12.1 Access to Source Data

According to ICH-GCP and the applicable German laws, the investigator must permit all authorized third parties access to the trial site and the medical records of the trial subjects (source data). These include the clinical monitors, auditors and other authorized employees of the sponsor, as well as members of the local or competent authorities. All these persons are sworn to secrecy.

12.2 Clinical Monitoring

Monitoring on site will be performed by the ZKS Leipzig - KKS. The monitoring includes pre-study visits before start of study in order to assess the participating trial sites.

The detailed planning and conduct of the monitoring will be based on the ZKS Leipzig monitoring SOPs and the study specific monitoring manual which will be prepared before start of the trial.

The investigator and key trial personnel must be available to assist the monitor during monitoring visits. The investigator must give the monitor access to relevant hospital or clinical records, to confirm their consistency with the CRF entries.

Initiation visits are planned in all participating sites prior to trial start. At this visit the study monitor will explain the protocol and case report forms (CRFs) with the investigators and their staff as well as the trial process in detail. In addition, extensive written guidance (working instructions) will be provided with the ISF (investigator site file).

A risk-based monitoring strategy will be implemented, using the risk-based approach proposed by the ADAMON project group. During trial conduct, central and statistical monitoring procedures will be combined with on-site monitoring visits in order to achieve high protocol compliance and data quality, as well as to ensure patients' safety and rights. The first monitoring visit at a site will be scheduled early after the inclusion of the site's first patient including respective documentation, checking protocol compliance and preventing further systematic errors due to misunderstandings. All trial sites will be visited regularly then. The frequency of further monitoring visits will depend on the trial site's recruitment rate and on whether problems have been detected with the site, either by prior on-site visits or by

central monitoring. However, trial sites will be visited at least every 12 months. During the visits, the monitor will

- Check informed consent forms of all patients enrolled
- Perform targeted source data verification for patients where the synopsis indicates possible derivations
- Perform source data verification of the key data (eligibility criteria, baseline parameters, G-CSF therapy delivery, ACLF complications, infections, subsequent ACLF episodes, adverse events) in a random sample of at least 20 % of the site´s patients (in addition to the targeted source data verification as described above)
- Discuss open queries raised by data management or safety personnel check and update the investigator site file if adequate resources available.

A last visit will be arranged for close out. This visit may be performed by telephone. Monitor and investigator will discuss all trial-related documents for archiving and the monitor will instruct the investigator about the archiving procedure and periods thereof.

12.3 Audits

In order to guarantee that the conduct of the study is in accordance with ICH-GCP and the national laws, the sponsor reserves the right to audit selected trial sites. The auditor will be independent from the staff involved in the proceedings of this clinical study.

The investigator agrees to give the auditor access to all relevant documents for review.

12.4 Inspections

According to German drug law (AMG) and the corresponding GCP-guidelines (GCP-V), inspections of the trial sites may be performed by the local or competent authorities at any time during or after completion of the trial.

The investigator agrees to give the inspectors access to all relevant documents for review.

12.5 Independent Supervision of the Trial

A Data Monitoring Committee (DMC) has been established. The DMC consists of two clinical and one non-clinical expert (biometrician). Members of DMC are named in the Section of Responsible Parties.

The DMC is independent of the study sponsor and the study organisers.

The DMC will meet (by telephone or face-to-face) on a regular basis (approx. every 12 months). Descriptive Analyses concerning enrolment, study conduct, protocol adherence and safety issues will be supplied, in strict confidence, to the DMC, together with any other analyses that the committee may request. In the light of these analyses, the DMC will advise the coordinating investigator on the further study conduct. In addition, the DMC will evaluate the results of the interim efficacy analysis. It will recommend to the coordinating investigator and the sponsor whether to continue, modify, or stop the trial.

Before start of the trial a charter for the DMC will be set up.

13 DATA PROTECTION AND CONFIDENTIALITY

Within this study personal data of the trial subjects (name, date of birth, address, and telephone number) are collected and stored only at the trial site. Only gender, year of birth and data regarding the therapy and the course of disease (see schedule of methods and procedures) will be collected in the CRF and database.

The investigators are obliged to keep all study data and information confidential and to use those data only in context with the persons involved in the trial conduct. Study material or information generated in this trial must not be made available to third parties, except for official representatives of the sponsor or regulatory authorities. Since in the course of the trial contact between the trial centre and the patients is necessary, the patients' full name, address and telephone number will be ascertained and stored at the trial site after obtaining written permission to do so. This information will be stored separately from the trial data.

All trial-relevant data will be processed electronically and handled strictly confidential. Subjects will be identified throughout documentation and evaluation by the individual patient number only, whereas all subject names will be kept secret by the investigator. The data will be stored and processed in pseudonymized form (i.e. without reference to the patient's name) with the aid of an identification number.

Data will be analysed at the Zentrum für Klinische Studien Leipzig - KKS. The written safety concept of this institution ensures amongst other things that data access is limited to authorized persons, that measures are taken to prevent loss of data and that the laws pertaining to data protection are observed. The data are protected from third party access and only members of the trial are permitted access. These members are sworn to secrecy.

In the event of withdrawal of consent, the necessity for storing data will be evaluated. Data not needed will be deleted immediately. Personal data will be stored in an anonymous manner after reaching the study end, if there are no other regulatory or contractual time periods for archiving.

13.1 Declaration regarding Data Protection

During data entry, processing and analysis in the Zentrum für Klinische Studien Leipzig – KKS, Universität Leipzig, Härtelstr. 16-18, 04107 Leipzig, all requirements of the data protection act will be taken into account. Access to the data is strictly limited to authorized persons. Data are protected against unauthorized access.

13.2 Declaration regarding the Pseudonymized Transfer of Personal Data

The sponsor certifies herewith that the transfer of pseudonymized personal data will take place according to the documentation and communication regulations in §§ 12 und 13 of the GCP-guidelines. Moreover, the sponsor certifies that trial participants who do not permit the transfer of data will not be admitted to the trial.

14 ADMINISTRATIVE AGREEMENTS

14.1 Adherence to the Protocol

In order to ensure comparable conditions during all stages of the trial and in the interest of valid statistical analysis, the investigators, the coordinating investigator or any other person involved in the trial conduct may not alter the study conditions agreed upon and set out in this protocol.

All protocol violations will be immediately reported to the coordinating investigator (directly or via ZKS Leipzig monitoring or management staff). Protocol violations (PV) are all deviations from the procedures outlined in this document.

All protocol violations will be documented and discussed with the responsible biometrician. Protocol violations are classified into major (i.e. leading to exclusion from the per protocol set) or minor. In section 8.5.1, a list of deviations classified as major can be found. This list will be updated during trial conduct, in the light of the major deviations actually occurred.

Classification of protocol deviations will be finished before data bank lock and start of the statistical analysis, and will be part of the final statistical analysis plan.

The investigator must ensure that the recorded data are documented as per protocol. Minor variations are a inevitably, but must be documented together with a justification.

The clinical trial described here will be conducted and analysed in accordance with local laws (AMG/ GCP-Verordnung) and ICH guidelines for Good Clinical Practice (GCP) and Declaration of Helsinki.

14.2 Funding and Insurance

The GRAFT-Study is funded by the German Science Foundation (Deutsche Forschungsgemeinschaft, DFG), Förderkennzeichen EN 1100/1-1.

Patients are insured at the insurance company HDI-Gerling Industrie Versicherung AG, Niederlassung Leipzig; Eisenbahnstr. 1-3; 04315 Leipzig.

The number of the insurance policy is: 28-138971-03302.

Furthermore, an accident insurance take out for patients with the number of insurance police: 28138971-03545.

Copies of both insurance policies and the insurance conditions will be filed in the investigators file.

14.3 Notification of the Local Authorities

Prior to enrolment of the first patient in the trial, the sponsor, his/her legal representatives/ contractors and all investigators and their deputies according to German drug law AMG § 67 (1) and the requirements of the GCP-V § 12 and 13 are responsible for notifying the local regulatory authority of their participation in the trial.

According to § 67 (3) AMG and §§ 12,13 GCP-V the sponsor, his/her legal representatives/ contractors and all investigators and their deputies are also responsible for notifying the local regulatory authority of amendments, premature termination of trial arms or of the whole study and the regular trial termination.

14.4 Publication Policy and Registration

The GRAFT-Study shall be published under the lead of the coordinating investigator together with contributing partners in a peer-reviewed journal, irrespective of the trial results. The publication policy will follow the recommendations of Good Scientific Practice (GSP) of the Deutsche Forschungsgemeinschaft (DFG, http://www.dfg.de) and will meet the criteria of the International Committee of Medical Journal Editors (http://www.icmje.org).

The scientific add-on projects will be published under the indicated respective head of project in coordination with the coordinating investigator, meeting the standards of GSP as mentioned above.

Additional projects not predefined in this protocol might arise during the study. Results of those projects might be published only after publication of the predefined projects, unless the responsible authors are unable to complete the respective paper within one year after the end of the study. All not predefined projects will require approval by the coordinating investigator.

These projects, too, shall be published by meeting the standards of GSP as mentioned above.

All additional publications must refer to the GRAFT-Study and to the appropriate funding organisation.

Prior to study start, the clinical trial will be registered in a public trial registry (e.g. ClinicalTrials.gov).

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16 PROTOCOL SIGNATURES

Confirmation of the Final Protocol

We hereby certify that this is the final version 4.0 of the protocol:

Authorized representative of the sponsor = Coordinating investigator:	27.07.2018 Date	[redacted] Signature
Biometrician:	27.07.2018 Date	

17 PROTOCOL AGREEMENT

Herewith I declare that I have read and understood the present protocol and agree to honour each part of it. I will ensure that all the patients enrolled in the trial by my site will be treated, observed and documented in accordance with this protocol. I will ensure that all persons assisting with the study under my supervision are adequately informed about the protocol, the investigational product and their duties.

Date: _____

Signature of Investigator: _____

Affiliation/address (stamp): _____

_____

_____

_____

18 APPENDIX

18.1 Classification of Adverse Events

18.1.1 Degree of Severity

The degree of severity of an Adverse Event will be determined in accordance with the definitions in 7.1 and 0.

18.1.2 Assessment of Intensity

The assessment of the intensity accords with CTCAE V4.0

Mild Adverse Event	• asymptomatic or mild symptoms; • clinical or diagnostic observations only; • intervention not indicated.
Moderate Adverse Event	• minimal, local or noninvasive intervention indicated; • limiting age-appropriate instrumental ADL*[3].
Severe Adverse Event	• medically significant but not immediately life-threatening; • hospitalization or prolongation of hospitalization indicated; • disabling; • limiting self care ADL**
Life-threatening Adverse Event	• Life-threatening consequences; • urgent intervention indicated
Death related to Adverse Event	

18.1.3 Determining the Causal Relationship

The investigator/ the deputy or the authorized medical staff must assess whether or not the Adverse Event is causally related to the administration of the trial medication. The following classification is to be used.

- Reasonable possibility
- No reasonable possibility

A reasonable possibility exists, if one of the following WHO-UMC criteria is met:

- occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be

[3] **Activities of Daily Living (ADL):**
*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

- with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfil this definition.
- with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
- more data is essential for a proper assessment or the additional data are under examination
- cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified

No reasonable possibility exists, if the following WHO-UMC criterion is met:

- with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

18.1.4 Expected/Unexpected

Adverse Events are unexpected if they do not occur in the manner or with the intensity described in the *SmPC/Investigator's Brochure* (see investigator's files).

18.1.5 Outcome of an Adverse Event

The outcome of an Adverse Event is classified as follows:

- recovered/resolved
- recovering/resolving
- not recovered/not resolved
- recovered/resolved with sequelae
- fatal*
- unknown

*Note: A patient's death is not in itself an event, but the consequence of one. The event that led to the patient's death must be documented completely and reported even if death occurs four weeks after stopping medication and independent of whether or not there is a relation to the therapy or not.

18.2 Definitions

Organ/system	Subscore = 1	Subscore = 2	Subscore = 3
Liver	Bilirubin <6 mg/dl	Bilirubin ≥6 mg/dl and <12 mg/dl	Bilirubin ≥12 mg/dl
Kidney	Creatinine <2 mg/dl	Creatinine ≥2 mg/dl and <3.5 mg/dl	Creatinine ≥3.5 mg/dl or renal replacement
Brain (West-Haven grade for HE*)	Grade 0	Grade 1-2	Grade 3-4**
Coagulation	INR <2.0	INR ≥2.0 and <2.5	INR ≥2.5
Circulatory	MAP ≥70 mmHg	MAP <70 mmHg	Use of vasopressors
Respiratory PaO_2/FiO_2 or SpO_2/FiO_2	 >300 or >357	 ≤300 and >200 or >214 and ≤357	 ≤200# or ≤214#

The shaded area describes criteria for diagnosing organ failures.
*HE, hepatic encephalopathy; FiO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; SpO_2, pulse oximetric saturation.
**Patients submitted to Mechanical Ventilation (MV) due to HE and not due to a respiratory failure were considered as presenting a cerebral failure (cerebral subscore = 3).
#Other patients enroled in the study with MV were considered as presenting a respiratory failure (respiratory subscore = 3).

Table 2: Organ failures are defined according to the CLIF-C OFs [Jalan 2014]

18.3 Acronyms

ACLF	Acute-on-chronic liver failure
AE	Adverse Event
AMG	Arzneimittelgesetz
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
BMSC	Bone marrow derived stem cells
BOB	Bundesoberbehörde
EK	Ethikkommission
FAS	Full analysis set
GCP	Good Clinical Practice
GCP-V	GCP-Verordnung
ICH	International Conference on Harmonisation
IFN	Interferon
OLT	Orthotopic liver transplantation
SAE	schwerwiegendes unerwünschtes Ereignis (serious adverse event)
SAR	schwerwiegende Nebenwirkung (serious adverse reaction)
SIRS	Systemisches inflammatorisches Response-Syndrom
SUSAR	Unerwartete, schwerwiegende Arzneimittelnebenwirkung (suspected unexpected serious adverse reaction
WHO-UMC	World Health Organization – Uppsala Monitoring Centre