Summary of the Trial Report
[Synopsis according to ICH E3]

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

A prospective, open, randomized, controlled multicentre trial

GRAFT-Trial

Name of Finished Product/Name of Active Substance:
Ratiograstim/Filgrastim (rG-CSF)

Indication/Diagnosis:
Acute-on-chronic liver failure (ACLF)

EudraCT-Number:
2015-002212-32

ClinicalTrials.gov – Number:
NCT02669680

Date of report: 12.04.2021
Version: final 2.0

Trial start: 01.03.2016
End of Trial: 17.03.2020

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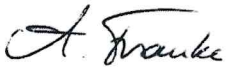
Signatures

The signing authors approve the report presented here by their signature. The described clinical trial was conducted according to the Declaration of Helsinki, Good Clinical Practice (GCP) as well as the applicable statutory provisions.

Legal representative of the sponsor and coordinating investigator	12. April 2021 Date	 Signature
Biometry	12.04.2021 Date	 Signature
Project management	12.04.2021 Date	 Signature

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1 Name of the Sponsor

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2 Name of Finished Product	3 Name of active Ingredient
Ratiograstim	Filgrastim (rG-CSF)

4 Individual trial table

not applicable

5 Title of Trial

Granulocyte colony stimulating factor (G-CSF) to treat acute-on-chronic liver failure: A multicentre randomized trial: final 4.0; 2018-07-26
including

- amendment 01; final 1.0 / 2016-06-21,
- amendment 02; final 1.0 / 2016-11-29 and
- amendment 03; final 1.0 / 2018-07-27

Relevant changes at trial protocol:

➢ **with amendment01**
Exclusion criteria
Old Text:
Septic shock as defined by ACCP/SCCM consensus [Bone 1992]
New Text:
Septic shock, defined by the following symptom complex: bacteraemia AND SIRS AND shock
Documentation and Reporting of AE/SAE

New Text / Addition:
Furthermore the following exceptions are defined in case of an orthotopic liver transplantation (OLT):
- If an OLT occurs before day 28, (S)AEs have to be documented **up to 3 days** after the last injection of G-CSF in the experimental arm,
- OLT itself must **not** be reported as (S)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** (S)AE reporting necessary
- relationship with G-CSF possible -> (S)AE reporting necessary
- relationship with G-CSF or OLT inconclusive -> (S)AE reporting necessary

➢ **with amendment02**

→ no changes at the trial protocol

➢ **with amendment03**
Due to poor recruitment, the DFG (public funding of the trial) approved a prolongation of the recruitment period by 12 months, resulting in following time lines:
First patient in to last patient out (months): 48
Duration of the entire trial (months) including preparation and analysis: 60
Recruitment period (months): 36

Based on current safety information (Rote Hand Brief 2018-06-25) on Filgrastim the following exception from SAE reporting was added:
Any diagnostically confirmed aortitis causes a SAE report even if it does not meet the SAE criteria.

6 Investigator		**7 Trial Centres**
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8 Publications

Engelmann, C. et al. Granulocyte-Colony Stimulating Factor (G-CSF) to treat acute-on-chronic liver failure a multicenter randomized trial (GRAFT study): Interim analysis of the first randomized European trial. Hepatology; 70(S1):1476

Engelmann, C. et al. Granulocyte-Colony Stimulating Factor (G-CSF) to treat acute-on-chronic liver failure a multicenter randomized trial (GRAFT study): submitted February 2021

9 Studied period (in years)

Date of first enrolment: 01.03.2016

Date of last completed: 17.03.2020

In April 2019 the recruitment was terminated prematurely based on conditional power analyses which was performed in parallel to the scheduled interim analysis (according to trial protocol). The final decision regarding the premature termination due to futility was made by the authorized representative of the sponsor after intensive discussion of the results of interim analysis with the biometrician and members of DMC.

10 Phase of Development

was not named in trial protocol (corresponds to phase 2b)

Commercially available, approved medication will be used.

11 Objectives

Primary objective:

Since the prognosis of ACLF is still poor, only a limited number of ACLF patients and mostly only those with a very poor prognosis may receive an organ due to shortage of donor organs in Germany. 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]. In two small randomized trials the administration of G-CSF showed a beneficial effect on the outcome after ACLF [Garg 2012, Duan 2013]. The safety profile is well known, with minor side effects like headache, fever or bone pain. In the context of efficacy evaluation of G-CSF, 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].The primary endpoint of the Graft trial is therefore chosen as transplant-free survival (TFS) up to 90 days, with death and liver transplantation (OLT) counting as event.
It should be assessed whether there is a difference in the transplant-free survival up to 90 days after randomization between the two trial 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)

Furthermore, both TFS and overall survival were analyzed until the end of regular observation at day 360 = End of trial.

Secondary objectives:

To assess group-differences regarding

- Occurrences of complications of ACLF: hepatorenal syndrome (HRS), ascites, hepatic encephalopathy (HE)), variceal bleeding
- Occurrences of bacterial infections (proven infection necessitating systemic use of antibiotics)
- Liver function during the course of treatment and follow-up (by MELD score and Child-Pugh score)
- Duration of the initial hospital stay

12 Methodology

GRAFT was a randomized, open-label 2-armed multi-centre trial. Patients were randomized 1:1 either to standard medical care plus G-CSF (Filgrastim) or standard medical treatment (SMT) alone. Randomisation of patients into both treatment arms was performed centrally by the ZKS Leipzig. The randomisation procedure was computer-assisted via internet, based on a minimisation algorithm as described in ZKS standard operating procedures for biometrical procedures and included a random component of 20% to avoid a completely deterministic allocation. A block randomisation list with variable lengths of 2, 4, or 6 was used in case of technical/ connectivity problems of the centres and done by ZKS data management.
Because of a) various discrepancies in available and recommanded web-based calculation schemes of ACLF grade, b) insufficient familiarity of the centres in using these websites and c) the rather high sample size, we abstained from the initially intended stratification by ACLF grade, but collected the all essential data and calculated the ACLF grades per patient and visits (in case of availability of all detail data) to allow analyses with consideration of the disease's severity at the start of intervention.

Descriptive analyses concerning enrolment, trial conduct, protocol adherence and safety issues were regularly reviewed by a Data Monitoring and Safety Committee (DMSC). It recommended to the coordinating investigator and the sponsor whether to continue, modify, or stop the trial.

13 Number of patients (planned and analysed)

Planned number: 292 patients (146 patients per treatment group)

Registered/screened subjects: 180

Recruited subjects: 176 patients (88 patients per treatment group)

Analyzed patients: 176 patients (88 patients per treatment group)

Drop-outs: 28 (censored observations without endpoint before Day 360)

For details see the CONSORT flow diagram in appendix 21.1.

14 Diagnosis and main criteria for inclusion

Indication	Acute-on-chronic liver failure (ACLF)
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

15 Information on the Test Product

Dose:
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).
Mode of Administration:
G-CSF will be injected subcutaneously.

Batch numbers:
Commercially available, approved medication will be used.
21 different batches of G-CSF medication (Ratiograstim) were applied in the course of trial.

Batch number	Frequency	Percent
507597	12	1,6
HB6840	1	,1

HH5478	1	,1
Neupogen	4	,5
P43409	30	4,0
R235398	4	,5
R28152	1	,1
R35398	112	14,9
R42207	31	4,1
S04587	16	2,1
S07597	125	16,6
S28032	22	2,9
S28037	34	4,5
S39844	64	8,5
T07028	20	2,7
T25193	16	2,2
T25194	20	2,7
U01426	25	3,3
U01540	195	25,9
U19966	9	1,2
UO1540	11	1,5
Total	753 [1]	100,0

16 Duration of Treatment

Overall, twelve doses of G-CSF were planned to be administered subcutaneously daily on days 0-4, thereafter every 3rd day over 26 days (days 7, 10, 13, 16, 19, 22, 25).

17 Reference Therapy

Standard care of acute-on-chronic liver failure

18 Criteria for Evaluation

18.1 Efficacy

Primary endpoint: transplant –free survival until day 90 post randomisation which corresponded to visit 6 (TFS D90)

Secondary endpoints; occurrences until day 360= end of observation:

1. TFS
2. Overall survival

Complications with observed occurrences until day 360= end of observation:

3. Hepatorenal syndrome (HRS)
4. Ascites (ASC)
5. Hepatic encephalopathy (HE)

[1] in total: 753 G-CSF applications within the GRAFT trial

6. Bacterial infections

Liver scores
7. MELD score during the course of observation until V6:
8. Child Pugh score during the course of entire observation:

18.2 Safety

- SAE per arm
- SAR
- Deaths per arm (by MedDRA SOC/ preferred terms)
- AE per arm, with numbers reported per arm; *with reference to the reporting guidelines of the EUDRA-CT database (which allow to concentrate on those AE occurred more often than in single cases) we report all AE Preferred Terms which occurred at least in at least 8 events during the entire course of trial (> 4%).*

19 Statistical Methods/analysis procedures

The **primary efficacy analysis** was performed by Cox regression within the full analysis set (FAS) of patients. The proportional hazard assumption was justified within the data. Hazard ratio for treatment effect and 95% confidence intervals was presented. Times to events were described by Kaplan-Meier curves.
Rates of complications, infections and further binary outcomes were be compared by chi-squared test. The course of the MELD score until day 90 per arm was described and repeated measures analyses performed to identify possible group differences.
Exploratory subgroup analyses focused on the type of underlying liver disease (alcoholic hepatitis, viral hepatitis see publication).
Safety issues were presented in Annual Safety Reports (ASR) with the last report from January 2021 after the end of the (S)AE report reriod.
Details of statistical analyses were be pre-specified in statistical analysis plans for the scheduled interim and the final analysis according trial protocol.

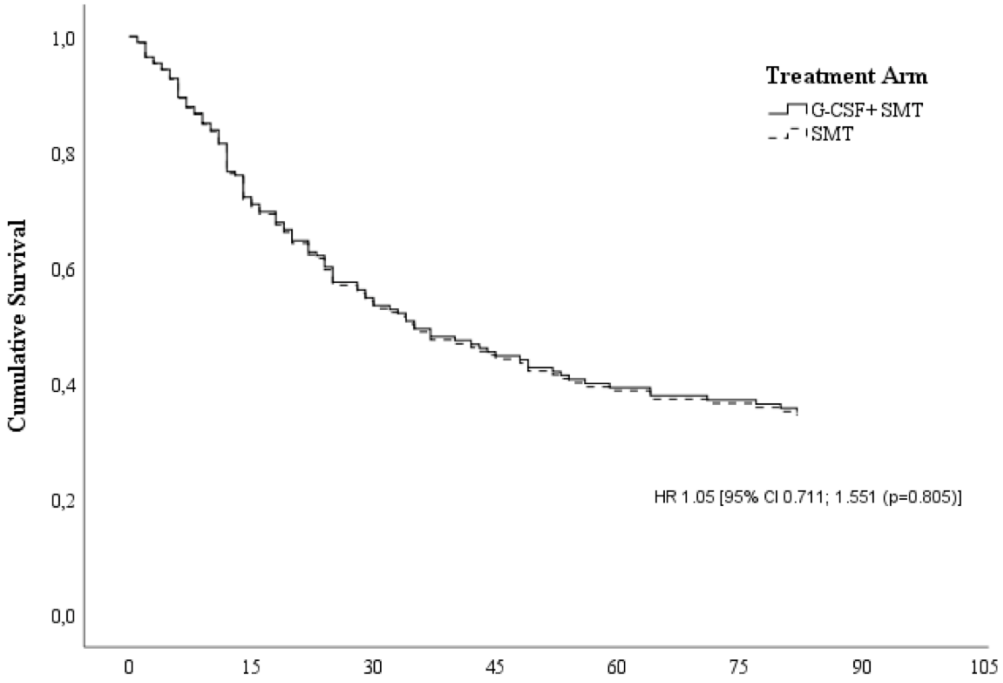
On recommendation of the Data Monitoring and Safety Committee and after decision of the coordinating investigator the trial's recruitment was prematurely terminated due to futility based on conditional power analyses which were done in parallel to the scheduled interim analyses at the time when the primary endpoint was available in about half of the planned sample.
We calculated a conditional power of 1-β=0.0266 for transplant-free survival after 90-days, and 0.0046 for overall survival.

20 Summary/Conclusion

20.1 Efficacy results

Primary endpoint TFS D90: p=0.805;
HR [95% confidence interval], from cox regression: exp(B)=1.05 [0.711,1.551].
The median time for transplant-free survival was 35 days (95%CI 19.8; 50.2) in the G-CSF+SMT group and 34 days (95%CI 9.9; 58.1) in the SMT group.

No significant arm differences were found between G-CSF plus standard medical treatment and standard medical treatment alone.



Patients at risk

G-CSF + SMT	**88**	**61**	**42**	**35**	**29**	**28**	**27**
SMT	**88**	**54**	**43**	**35**	**31**	**28**	**26**

Figure 1: 90-day transplant-free survival in ITT cohort

Major secondary endpoints, occurrences until day 360 = end of observation:

1. TFS D360: p=0.992; HR=exp(B)=1.05 [0.711,1.551] from cox regression,
   HR [95% confidence interval], from cox regression: exp(B)=0.998 [0.697,1.430]
2. Overall survival: p=0.768; HR=exp(B)=1.058 [0.711,1.551] from cox regression,
   HR [95% confidence interval], from cox regression: exp(B)=1.058 [0.727,1.548]

3. MELD score during the course of observation until V6:
   - Complete courses (n=17 G-CSF vs n=18 control arm):
     $p_{Main\ effect}$ =0.884); $p_{time*arm\ interaction}$)=0.141;
     with G-CSF arm presenting with marginally higher mean estimation of 0.158 (95%CI: -2,037; 2.353)
   - with last values imputed due to early deaths (n=87 G-CSF vs n=86 control arm):
     $p_{Main\ effect}$ =0.080); $p_{time*arm\ interaction}$)=0.698;
     with the G-CSF arm presenting with marginally higher mean estimation of -0.131 (95%CI: -2,780; 0.160)

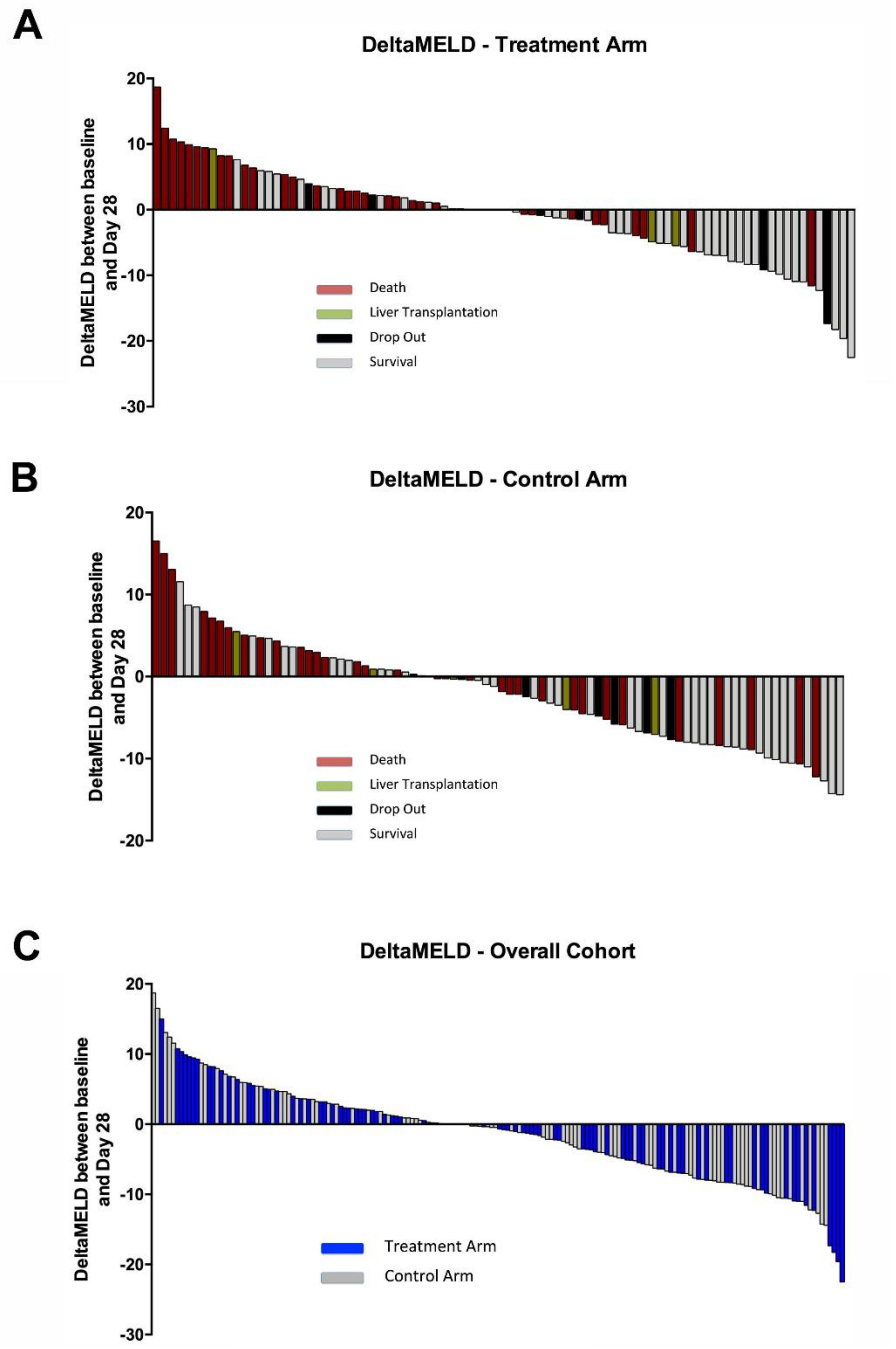


Figure 2: Development of MELD during the treatment course

4. Child Pugh score during the course of entire observation:
   without significant arm differences at baseline: $p_{chi^2}=0.307$
   without significant arm differences at V5: $p_{chi^2}=0.213$ resp. $p_{chi^2\ lin}=0.940$
   without significant arm differences at V6: $p_{chi^2}=0.152$ resp. $p_{chi^2\ lin}=0.366$
   without significant arm differences at V8=EoS: $p_{chi^2}=0.287$ resp. $p_{chi^2\ lin}=0.650$

5. Length of initial hospitalization: in 26 G-CSF and 22 SMT patients a discharge from hospitalisation was observed after a mean duration of 25.9 (SD 15.5) d vs 19.0 (SD 13.8) d post randomisation. Although a marginally higher proportion was reported under G-CSF patients this difference was not significant (p=0.498).

In none of the (further) secondary endpoints a significant group difference was observed, see also main publication.

In conclusion, this multi-centre randomised trial failed to demonstrate an improvement of transplant-free or overall survival in patients with ACLF. G-CSF should not be used outside of controlled clinical trials for ACLF patients.

20.2 Safety results

The latest ASR with its cut-off date from December 2019 comprised all SAE/SAR reported within the GRAFT trial. The end of the SAE reporting period for the LPI was far before that date, beside from requested SAE reports of new malignancies which should be reported until end of observation according to trial protocol. However, none did occur in the trial's survivors until the end of observation.
Sixty-one SAE reports (cases) of 54 patients from the G-CSF+SMT group, as well as 57 SAE reports of 47 patients from the SMT group were provided by the centres, including 37 and 36 deaths during the SAE reporting period, respectively. After splitting the reported cases into MedDRA-codable SAE terms a total of 97 resp. 109 SAE events per arm were observed, see the following tables which presents the type and frequency as per MedDRA System Organ CLASS, Preferred Terms and arm.

SOC	Preferred Term	G-CSF+ Std.-ther.	Standard therapy	Total
Blood	Anaemia	0	2	2
	Coagulopathy	1	0	1
	Total	1	2	3
Card	Atrial flutter	1	0	1
	Cardiovascular insufficiency	0	1	1
	Pulseless electrical activity	1	0	1
	Right ventricular failure	0	1	1
	Total	2	2	4
Gastr	Acute abdomen	0	1	1
	Ascites	2	1	3
	Dieulafoy`s vascular malformation	1	0	1
	Duodenal ulcer perforation	1	0	1
	Gastrointestinal haemorrhage	6	4	10
	Haemoperitoneum	0	1	1
	Haemorrhoidal haemorrhage	1	0	1
	Ileus	0	1	1
	Intestinal haemorrhage	0	1	1
	Intestinal ischaemia	1	0	1
	Intestinal varices haemorrhage	0	2	2
	Oesophageal varices haemorrhage	2	0	2
	Upper gastrointestinal haemorrhage	0	2	2
	Total	14	13	27
Genrl	General physical health deterioration	1	1	2
	Multiple organ dysfunction syndrome	11	9	20
	Systemic inflammatory response syndrome	0	1	1
	Ulcer haemorrhage	0	1	1
	Total	12	12	24

Hepat	Acute on chronic liver failure	8	7	15
	Hepatic cirrhosis	0	1	1
	Hepatic failure	5	6	11
	Hepatic function abnormal	2	0	2
	Hepatorenal syndrome	6	4	10
	Total	21	18	39
Infec	Abdominal wall infection	1	0	1
	Infection	0	2	2
	Influenza	1	0	1
	Peritonitis	1	0	1
	Peritonitis bacterial	1	3	4
	Pneumonia	1	4	5
	Pulmonary sepsis	0	1	1
	Sepsis	3	3	6
	Septic shock	1	1	2
	Urosepsis	0	1	1
	Total	9	15	24
Inj&P	Post procedural haemorrhage	0	1	1
	Subdural haematoma	1	1	2
	Total	1	2	3
Inv	Blood sodium decreased	0	1	1
	Transaminases increased	1	0	1
	Total	1	1	2
Metab	Acidosis	0	2	2
	Fluid overload	1	0	1
	Hyponatraemia	1	0	1
	Lactic acidosis	2	0	2
	Total	4	2	6
Neopl	Choroid melanoma	1	0	1
	Total	1	0	1
Nerv	Cerebral haemorrhage	1	1	2
	Coma hepatic	0	1	1
	Disturbance in attention	1	2	3
	Encephalopathy	1	0	1
	Generalised tonic-clonic seizure	1	0	1
	Hepatic encephalopathy	5	10	15
	Seizure	1	0	1
	Status epilepticus	1	0	1
	Subarachnoid haemorrhage	0	1	1
	Total	11	15	26
Renal	Acute kidney injury	1	1	2
	Anuria	1	0	1

	Renal failure	1	1	2
	Renal impairment	1	1	2
	Total	4	3	7
Resp	Acute respiratory distress syndrome	1	1	2
	Asphyxia	1	0	1
	Aspiration	1	0	1
	Epistaxis	0	1	1
	Pleural effusion	1	2	3
	Pulmonary oedema	1	2	3
	Respiratory failure	3	6	9
	Total	8	12	20
Surg	Endotracheal intubation	0	1	1
	Haemodialysis	1	0	1
	Liver transplant	0	1	1
	Portal shunt procedure	1	0	1
	Resuscitation	2	1	3
	Thoracic cavity drainage	1	0	1
	Total	5	3	8
Vasc	Bleeding varicose vein	0	1	1
	Circulatory collapse	1	2	3
	Haemorrhage	0	1	1
	Hypotension	2	2	4
	Shock haemorrhagic	0	3	3
	Total	3	9	12
Total	Total	97	109	206

Table 1: SAE by MedDRA SOC and Preferred Term per arm

The following table presents the type and frequency of SAR during the course of the trial.
In the G-CSF+SMT group, seven possibly G-CSF-related serious adverse reactions in four patients were assessed. In eight patients (9.1%), G-CSF therapy had to be transiently stopped as the leucocyte count was exceeding 70 x 10^9/L. Therefore, G-CSF therapy may be associated with a possible risk of drug related adverse reactions.

Acute kidney injury	1
Hepatic failure	1
Multiple organ dysfunction syndrome	2
Peritonitis bacterial	1
Respiratory failure	1
Transaminases increased	1
Total	7

Table 2: SAR by MedDRA Preferred Term (all in G-CSF arm)

Patients' causes of death were reported in the following table, with MedDRA Preferred Terms if occurred within the (S)AE reporting period (28 days after randomisation).

		treatment arm (acc. to rando)		
		G-CSF+ Std.-ther.	Standard therapy	total
Cause/s of death (PT), cumulative per patient	*no death reported*	*30*	*33*	*63*
	death after end of SAE reporting period	*20*	*17*	*37*
	death after OLT; no SAE report required	*1*	*2*	*3*
	Acute kidney injury	0	1	1
	Acute on chronic liver failure	8	5	13
	Acute on chronic liver failure, Multiple organ dysfunction syndrome	0	1	1
	ARDS, Pneumonia	0	1	1
	Cerebral haemorrhage	1	1	2
	Circulatory collapse	1	0	1
	Circulatory failure, Acute on chronic liver failure	0	1	1
	Coma hepatic	0	1	1
	Death	1	0	1
	Gastrointestinal haemorrhage	0	2	2
	Haemorrhage	1	0	1
	Hepatic cirrhosis	0	1	1
	Hepatic failure	5	5	10
	Hepatorenal syndrome	3	0	3
	Intestinal ischaemia	1	0	1
	Liver failure, Circulatory collapse	1	0	1
	Multi organ failure, Septic shock	0	1	1
	Multiorgan failure, Acute on chronic liver failure	0	1	1
	Multiorgan failure, Cerebral haemorrhage	1	0	1
	Multiorgan failure, Urosepsis	0	1	1
	Multiple organ dysfunction syndrome	7	6	13
	Oesophageal varices haemorrhage	1	0	1
	Peritonitis bacterial	0	2	2
	Respiratory failure	1	1	2
	Respiratory failure, Acute kidney injury	1	0	1
	Respiratory failure, Influenza	1	0	1
	Right heart failure, Transplant failure	0	1	1
	Sepsis, Multiple organ dysfunction syndrome	1	0	1
	Septic shock	1	0	1
	Septic shock, Peritonitis bacterial	1	0	1
	Shock haemorrhagic	0	2	2
	Shock haemorrhagic, Gastrointestinal haemorrhage	0	1	1
	Shock haemorrhagic, Hepatic failure	0	1	1
	total	88	88	176
		100,0%	100,0%	100,0%

Table 3: Causes of patients' deaths from SAE reports (if applicable)

In total, 403 adverse events were reported from in total 80 patients from the G-CSF treatment cohort and 354 in 78 patients from the SMT group.
In accordance with the EudraCT database (see also section 18.2) the following table comprises all AE with MedDRA Preferred Terms occurred more often than 7 times during the entire course trial. This refers to 175 (of 403) resp. 163 (of 354) AE per arm.

		treatment arm (acc. to rando)		
		G-CSF+ Std.-ther.	Standard therapy	Total
AE PT-Term	Acute on chronic liver failure	9	8	17
	Anaemia	4	7	11
	Ascites	17	13	30
	Gastrointestinal haemorrhage	16	12	28
	Hepatic encephalopathy	21	25	46
	Hepatic failure	6	6	12
	Hepatorenal syndrome	19	18	37
	Hypokalaemia	9	12	21
	Hypotension	6	7	13
	Leukocytosis	8	1	9
	Multiple organ dysfunction syndrome	11	9	20
	Nausea	9	3	12
	Peritonitis bacterial	15	8	23
	Pneumonia	8	12	20
	Respiratory failure	5	8	13
	Sepsis	4	7	11
	Urinary tract infection	8	7	15
Total		175	163	338

Table 4: More frequently (>7 times) reported AE (per MedDRA Preferred Term and arm)

20.3 Conclusions

Our trial was the first large multi-centre trial that evaluated G-CSF as a potential novel therapy for patients with ACLF. Despite G-CSF having immune-modulatory and pro-regenerative capacities, our trial confirmed the overall dismal prognosis of ACLF and failed to demonstrate G-CSF superiority over standard medical therapy. G-CSF was associated with few but potentially drug related serious adverse reactions and should not be used outside clinical trials to treat ACLF.

21 Appendix

21.1 CONSORT Flow Diagramm

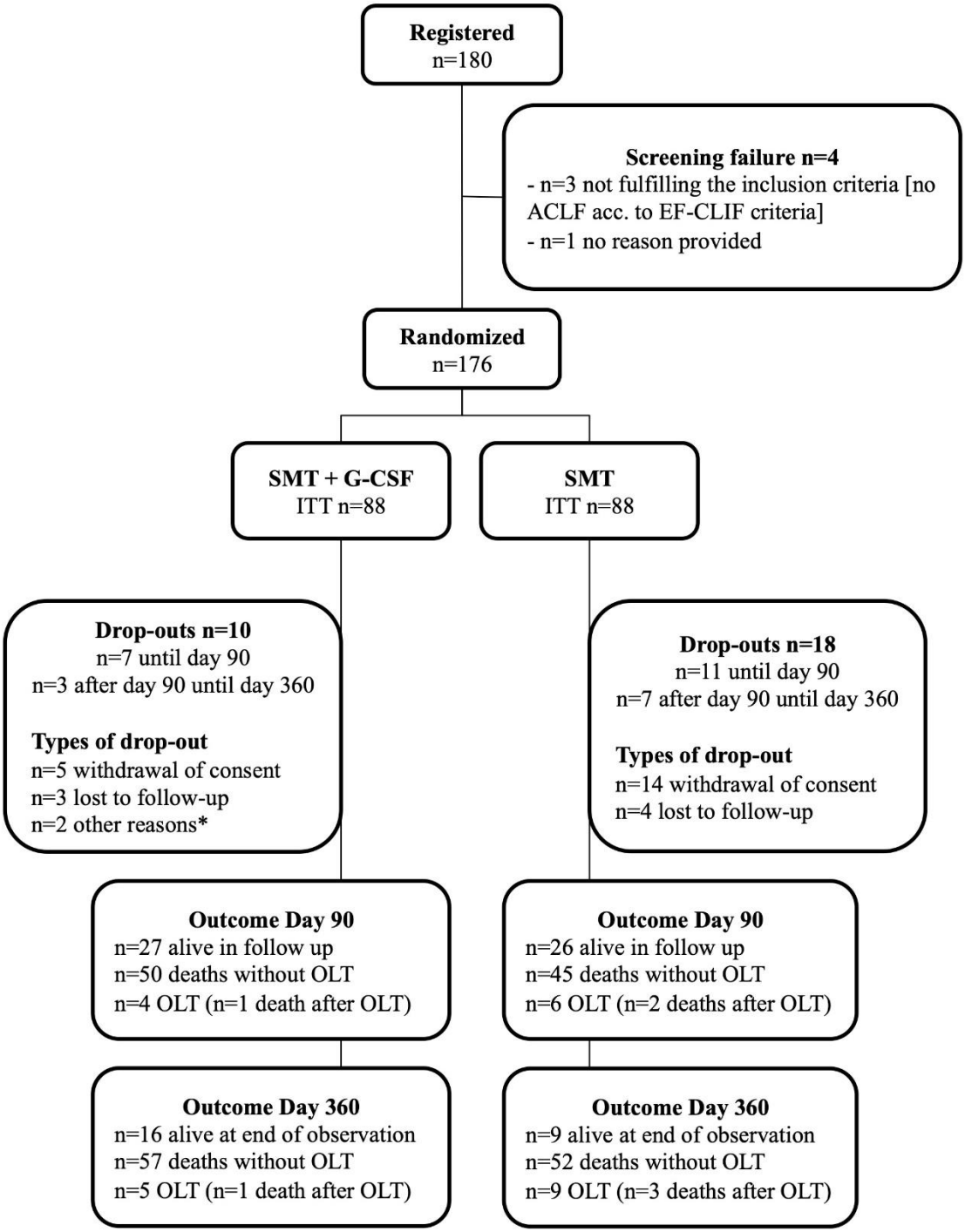


Figure 3: CONSORT flow chart of patients