DECISION

DEcompensated CIrrhoSIs: identification of new cOmbiNatorial therapies based on systems approaches

H2020 – 847949

D4.1 Generation of rat models of acute decompensation of cirrhosis developing ACLF

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Abbreviations

ACLF	Acute-on-chronic liver failure
AD	Acute decompensation
ASH	Alcoholic steatohepatitis
BDL	Bile-duct ligation
CCl_4	Carbon Tetrachloride
CLP	Cecal ligation and puncture
EtOH	Ethanol
LPS	Lipopolysaccharide (found in the outer membrane of Gram-negative bacteria)
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
PBS	Phosphate-buffered saline
SD	Sprague Dawley
TNI	Transnasale inocculation
WD	Western Diet

Contributors

Contributor	Contribution	Estimate of person-months
GUF, set up rat models of acute decompensation of cirrhosis developing	CCl_4 Control CCl_4 + 2 x LPS CCl_4 + Alcoholic binge CCl_4 + TNI BDL Control BDL + 2 x LPS BDL + Alcoholic binge BDL + TNI CCl_4 + EtOH Control CCl_4 + EtOH + 2 x LPS CCl_4 + EtOH + Alcoholic binge CCl_4 + EtOH + TNI CCl_4 + Western diet Control CCl_4 + Western diet + 2 x LPS CCl_4 + Western diet + TNI	4x 1 PM Postdoc 9x 1 PM Phd Student
FCRB, set up rat models of acute decompensation of cirrhosis developing	CCl_4 Control Cecal ligation and puncture control CCl_4 + Cecal ligation and puncture CCl_4 + Western diet Control Western diet Control CCl_4 + Western diet + Cecal ligation and puncture	3x 1 PM Postdoc 3x 1 PM Laboratory Technician

Executive Summary

In work package 4, GUF and FCRB generate rat models of acute decompensation of cirrhosis developing ACLF. Since the animal facility at INSERM had to be transferred due to renovation, which would have delayed the animal experiments, a discussion between partners led to the decision that INSERM will not carry out animal experiments within DECISION. Instead, FCRB and GUF generated the animal models, while INSERM will focus on the characterization of the different animal models of ACLF.

Subsequently, GUF, INSERM and FCRB will characterize the organs obtained from the rat models of acute decompensation of cirrhosis developing ACLF to identify the best models reflecting the human ACLF disease. A combinatorial therapy identified by WP3 will be tested in the best rodent model of acute decompensation with ACLF.

Four different models of liver cirrhosis are investigated in WP4:

- the bile-duct ligation (BDL) model resulting in cholestatic liver cirrhosis,
- the toxic (CCl_4) model,
- simultaneous CCL_4 treatments and ethanol administration, resulting in the alcoholic model (alcoholic steatohepatitis, ASH) and
- simultaneous CCL_4 treatments and high-fat western diet feeding, resulting in the non-alcoholic model (non-alcoholic steatohepatitis, NASH).

The cholestatic liver cirrhosis is induced by BDL operation and the toxic liver cirrhosis with CCl_4 treatments. In parallel to the CCl_4 treatment, ASH is induced by administering 16% ethanol in the drinking water. NASH is induced by feeding rats with a high fat western diet (WD) simultaneously with CCl_4 treatment.

When rats develop ascites as a definite sign of portal hypertension, the precipitating events to induce ACLF are performed: ACLF is induced by mimicking bacterial translocation, pneumonia or alcoholism to reflect the human ACLF disease. As such, the bacterial translocation will be mimicked in cirrhotic rats by repeated LPS injection in the tail vein. Pneumonia is induced by a transnasal stool inoculation. Alcoholism as a precipitating event inducing ACLF is modelled by binge alcohol intake via oral gavage in rats.
Since alcoholism is not a precipitating event in ACLF patients with a non alcoholic steatohepatitis (NASH) the alcohol binge drinking is not performed in NASH or CCl_4 treated rats, but is administered in the group of ASH rats, which receive ethanol in the drinking water and at the same time CCl_4 intoxication (please see table 1 for an overview).
The group of FCRB generated a sepsis model with an active infection as the precipitating event. This is done by a cecal ligature and puncture to mimic spontaneous bacterial peritonitis.

Task 1, i.e. setup of rat models of acute decompensation of cirrhosis developing ACLF, is successfully established.
The characterization and profiling of rat models of acute decompensation of cirrhosis developing ACLF is currently being analysed. Using these preliminary results, the best models and the best time points for ACLF inductions will be assessed and shared with the involved partners in WP4.

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Deliverable report

1 Animals

Common Sprague-Dawley (SD) rats are used for the experiments in WP4. Six week-old male Sprague-Dawley rats are housed in a 12:12 h light-dark cycle, with controlled temperature (21°C ± 2°C), and *ad libitum* access to standard rat chow/western diet and water/ethanol drinking water.

2 Induction of liver cirrhosis

2.1 Generation of bile duct ligated (BDL) rats developing ACLF

The cholestatic liver cirrhosis model is induced by BDL operation. When rats develop ascites as a definite sign of portal hypertension, the precipitating events to induce ACLF are performed.

2.2 Generation of toxic rat models developing ACLF

The toxic liver cirrhosis model in rats is induced by CCl_4 administration twice weekly for 14 weeks.

2.3 Generation of an alcoholic steatohepatitis model (ASH) developing ACLF

Cirrhosis in the alcoholic steatohepatits model (ASH) model is induced by CCl_4 administration twice weekly for 14 weeks. In addition, the rats have *ad libitum* access to an increasing amount of Ethanol starting at 4% of Ethanol in the drinking water for one week (week 0-1), followed by 8% Ethanol in the drinking water (week 1-2). The final concentration of ethanol in the drinking water is 16% after 3 weeks until the end of treatments.

2.4 Generation of a non-alcoholic steatohepatitis model (NASH) developing ACLF

Cirrhosis in the non-alcoholic steatohepatitis model (NASH) is induced by CCl_4 administration twice weekly for 14 weeks. In addition, the rats have *ad libitum* access to Western diet (containing 20.9% crude fat and 1.25% cholesterol; Ssniff Spezialdiäten GmbH). As a control to the NASH model, a separate group of rats received *ad libitum* access to the Western diet, resulting in the Non-alcoholic fatty liver disease (NAFLD) model.

2.5 Control rats

For each type of liver cirrhosis induction, control rats are run together with the rats in which cirrhosis is induced, both at GUF and at FCRB.

3 Precipitating events to induce ACLF

After the rats develop liver cirrhosis, ACLF is induced using different approaches as described below. The aim is to find the ACLF model that best represents the clinical disease profile. The characterisation of the established models is in progress and will be part of D4.2 "Characterization of the rat models of ACLF" (due: 31-03-2022).

3.1 Pulmonary infection by transnasale Inocculation (TNI)

0.1g of freshly collected stool is diluted in 2 mL sterile 0.9 % NaCl-solution (w/v) to prepare the TNI injection solution. The anaesthetized rats receive 50µL or 200µL of the TNI injection solution transnasally.

The assessment of organ function including systemic and portal hemodynamic measurements as well as the tissue aservation final experiment is performed 24h, 48h or 72h after TNI.

3.2 Lipopolysaccharide (LPS)

LPS is diluted in Aqua dest. to a final concentration of 50µg/mL. For ACLF induction, rats receive a single injection (120h) or two times injection (120h and 72h) before the final experiment. LPS is administered at a dose of 6.25µg/kg body weight.

3.3 Alcoholic binge (binge)

24, 72 or 120h before the final experiment, rats with BDL-induced cirrhosis or rats with ASH are administered a dose of 6g/kg body weight pure ethanol in an 32% Ethanol/0.9% NaCl solution (w/v) using an oral gavage.

3.4 Cecal ligation and puncture (CLP)

24, 48 or 72h before the final experiment, rats with CCl_4-induced cirrhosis and NASH rats undergo CLP surgery under general anaesthesia. Animals receive Buprenorfin (0.05mg/kg) as analgesic every 12h.

4 Tables and other supporting documents

Table 1: Overview of the planned and performed models and the respective executing institution.

GUF= Johann Wolfgang von Goethe Universität Frankfurt am Main, FCRB= Fundacio Clinic Per A LA Recerca Biomedica

			Precipitating event			
		Control	2 x LPS	Alcoholic binge	Cecal ligation and puncture	Pulmonary infection
Induction of liver cirrhosis	CCl4	GUF (n_1=10; n_2=4) FCRB (n_1=5)	GUF (n_1=10; n_2=4)		FCRB (n_1=7)	GUF (n_1=10; n_2=4)
	BDL	GUF (n_1=10; n_2=4)	GUF (n_1=10; n_2=10)	GUF (n_1=10; n_2=5)	GUF (n_1=10)*	GUF (n_1=10; n_2=4)
	CCl4 + Alcohol	GUF (n_1=10)	GUF (n_1=10)	GUF (n_1=10)	GUF (n_1=10)*	GUF (n_1=10)
	CCl4 + Western diet	GUF (n_1=10) FCRB (n_1=5)	GUF (n_1=10)		FCRB (n_1=7)	GUF (n_1=10)

n_1 is the total number of animals that entered the experiment, which are either still ongoing or have completed the experiment (i.e. they either died (which is an endpoint of the induction of ACLF) or from whom the organs were harvested). n_2 is the number of animals, from which organs were harvested. The discrepancy in the numbers of animals entering the experiment and those that reached the endpoint ('organs harvested') was caused by an increased mortality in specific models (similar to the human disease).

* For training and harmonization of the cecal puncture and ligation performance across sites a training by FCRB is mandatory. Due to travel restrictions during the Sars-Cov2 pandemic, GUF was not permitted to travel to FCRB for this training and is currently waiting for the travel permission.

Table 2: Detailed timeline of the planned rat models and the executing institutions.

Groups 1-16 GUF= Johann Wolfgang von Goethe Universität Frankfurt am Main,

Groups 17-21 FCRB= Fundacio Clinic Per A LA Recerca Biomedica

Group	Treatment	model	ACLF induction	ACLF model	Start	End	Animals started	tissue harvested
1	BDL	Cholestatic	-	-	July 2020	September 2020	10	4
2	BDL	Cholestatic	LPS	inflammation	July 2020	September 2020	10	10
3	BDL	Cholestatic	Alcoholic binge	Alcoholism	July 2020	September 2020	10	5
4*	BDL	Cholestatic	CLP	sepsis	pending	pending	-	-
5	BDL	Cholestatic	TNI	pneumonia	July 2020	September 2020	10	4
6	CCl_4	toxic	-	-	August 20	December 2020	10	4
7	CCl_4	toxic	LPS	inflammation	August 20	December 2020	10	4
8	CCl_4	toxic	TNI	pneumonia	August 20	December 2020	10	4
9	CCl_4 + WD	NASH	-	-	January 2021	April 2021	10	not finished
10	CCl_4 + WD	NASH	LPS	Bacterial translocation	February 2021	May 2021	10	not finished
11	CCl_4 + WD	NASH	TNI	pneumonia	January 2021	May 2021	10	not finished
12	CCl_4 + EtOH	ASH	-	-	February 2021	May 2021	10	not finished
13	CCl_4 + EtOH	ASH	LPS	inflammation	February 2021	May 2021	10	not finished
14	CCl_4 + EtOH	ASH	Alcoholic binge	Alcoholism	March 2021	May 2021	10	not finished
15*	CCl_4 + EtOH	ASH	CLP	sepsis	pending	pending	-	-
16	CCl_4 + EtOH	ASH	TNI	pneumonia	February 2021	May 2021	10	not finished
17	CCl_4 + WD	NASH	-	-	February 2021	May 2021	7	not finished
18	CCl_4 + WD	NASH	CLP	sepsis	February 2021	May 2021	7	not finished
19	WD	NAFLD*2	-	-	March 2021	June 2021	5	not finished
20	CCl_4	toxic	CLP	sepsis	January 2021	April 2021	5	not finished
21	CCl_4	Control	-	-	March 2021	June 2021	5	not finished

* For training and harmonization of the cecal puncture and ligation performance a training by FCRB is mandatory. Due to the Sars-Cov2 pandemic situation GUF is waiting for the travel permission.

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