Poster 21: LiSyM Midterm Evaluation 2018
Glahnemann/Lammert Group (Homburg)

Facts & Figures:
Area of Expertise: Gastroenterology, hepatology, molecular genetics, randomised controlled clinical trials
Laboratory methods: Genetic mouse models, histopathology, expression profiling by RT-PCR and ELISA, determination of hepatic collagen content by hydroxyproline assay and collagen area calculation, genotyping, sequencing, isolation of primary murine hepatic cells, cell culture

Translational studies and identification of prognostic markers
To identify prognostic markers, the diagnostic performance of three serum markers identified in the preclinical and in vitro models (cooperation with P4 Li. Koenig) was evaluated in terms of liver histology and stiffness in a total of N = 834 patients with chronic liver disease. A significant correlation was found between concentrations of three markers (HGF, GDF-15 and PLGF) and histopathology and liver stiffness (LS), suggesting this panel for non-invasive prediction of disease progression. Cut-offs for advanced liver fibrosis (IF2) were found to be 2.598 mg/mL, 1.584 mg/mL and 20.2 mg/mL for HGF, GDF-15 and PLGF, respectively (Table 3). An increase of odds ratios from 3.6 over 33.0 to 108.4 with incremental markers positive for LS ≥ 12.8 kPa (all P < 0.05) was confirmed by logistic regression analysis (Krawczyk et al. 2017).

Animal model of alcohol-induced ACLF
Since in INCA & GRAFT cohorts, alcoholic liver disease is the predominant aetiology of ACLF, we opted to extend the preclinical model with pre-existing liver injury due to deficiency of the hepatobiliary phosphatidylcholine transporter (Abcb4) mice, which shows spontaneous liver fibrosis, with ethanol challenge as standardized in the NIAAA protocol (Nat Protoc 2013). In the experimental set-up, ten (n=64) and 15 (n=64) week-old wild-type C57BL/6J and Abcb4 knock-out mice were either fed control or liquid ethanol diet (5% v/v), followed by an acute ethanol binge (5 mg/kg). Phenotypic characterization included hepatic expression profiling by qRT-PCR, ELISA, hepatic collagen contents and histopathology.

In a subgroup of patients (N=15), we compared LS to 1H-methacetin-based breath tests (LMAX) in patients undergoing portosystemic shunt intervention to reduce portal pressure (cooperation with pillar IV). LMax (which represents CYP1A2 activity) decreased significantly from 301±180 at baseline and 263±127 at intervention to 151±99 µg/kg/h at d14, pointing to a potential prognostic relevance of dynamic liver function tests to guide individualized treatment in decompensated liver cirrhosis (Maiiwosil et al. 2017).

Table 3. Determination of areas under ROC-curve of different serum markers according to histological fibrosis stages in the total cohort

| Serum marker | Area under ROC-curve AUC | 95% CI
|--------------|---------------------------|-----------
| HGF          | 0.84                      | 0.79-0.89 |
| GDF-15       | 0.81                      | 0.76-0.86 |
| PLGF         | 0.85                      | 0.81-0.89 |
| IF2 ≥ 3.6    | 0.83                      | 0.78-0.88 |
| IF2 ≥ 33.0   | 0.87                      | 0.83-0.91 |
| IF2 ≥ 108.4  | 0.94                      | 0.90-0.97 |

Table 4. Comparing expression levels of selected genes in control and ethanol-treated mice

| Gene        | Control (N=8) | Ethanol (N=8) | Fold change
|-------------|---------------|---------------|-------------
| Cebpa       | 1.20 ± 0.20   | 0.70 ± 0.10   | 0.58 ± 0.07 |
| Gadd153     | 1.50 ± 0.30   | 2.00 ± 0.40   | 1.33 ± 0.20 |
| Hnf4a       | 0.80 ± 0.10   | 1.20 ± 0.20   | 1.50 ± 0.30 |
| Il1r1       | 1.40 ± 0.20   | 2.00 ± 0.30   | 1.43 ± 0.21 |
| Tnf         | 0.60 ± 0.10   | 1.60 ± 0.30   | 2.67 ± 0.50 |

In a subgroup of ACLF patients (N = 86 until 01/2018), the impact of granulocyte-colony stimulating factor (G-CSF), 30 - 48 Mio. IU OD for one week and then every 3 days until d26 (Figure 3) is being assessed, together with systematic sampling of liver tissue and serum biomarkers. G-CSF mobilised stem as well as immune cells and improved liver function in preclinical trials, hence it represents a yardstick for future treatment design for patients with ACLF within the LiSyM network.

To assess future implications of our results in computational models, we summarized the current largest computational models of liver metabolism in a review in Hepatology, supported by LiSyM (Cvitanovic et al. 2017). Additionally, current evidence on predictive genomic markers in cholestatic liver diseases including our own prior data was summarized in another LiSyM-funded review (Reichert et al. 2017).

Publications:

Of note, non-linear regression analysis demonstrated that the hepatic repression of PNPLA3 resulted in a notable expansion of lipid droplet size after ethanol challenge, suggesting a key role for lipid remodelling in this setting (Figure 5).