Thieme

39. Jahrestagung der Deutschen Arbeitsgemeinschaft zum Studium der Leber

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Kongresspräsident:
Prof. Dr. Ali Canbay

Lecture Session I Basic Hepatology (Fibrogenesis, NPC, Transport) 27/01/2023, 13.25 pm – 14.10 pm, Lecture Hall

L1.01 A virtual liver twin to study the regeneration upon drug-induced injury

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DOI 10.1055/s-0042-1759891

This paper presents a mathematical mechanism-based model of the regenerating liver after drug-induced pericentral lobule damage resolving tissue microarchitecture. The consequence of alternative hypotheses about the interplay of different cell types on regeneration were simulated. Regeneration dynamics has been quantified by the size of the damage-induced dead cell area, the hepatocyte density and the spatial-temporal profile of the different cell types. We use deviations of observed trajectories from simulated system to identify branching points, at which the systems behavior cannot be explained by the underlying set of hypotheses anymore. Our procedure reflects a successful strategy for generating a fully digital liver-twin that, among others, permits to test perturbations from the molecular up to the tissue scale. The model simulations are complementing current knowledge on liver regeneration by identifying gaps in mechanistic relationships and guiding the system towards the most informative (lacking) parameters that can be experimentally addressed.

L1.02 Colitis protects from primary sclerosing cholangitis via bile acid synthesis suppression

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Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by chronic inflammation and progressive fibrosis of the biliary tree. PSC shows a striking association with inflammatory bowel disease with up to 80 % of patients suffering from concomitant colitis. Although this association has been known for decades, the molecular mechanisms by which intestinal

inflammation and the cholestatic liver may interact remain largely unknown. Here, we developed an IBD-PSC mouse model simulating the impact of colitis on bile acid metabolism and cholestatic liver injury. Unexpectedly, intestinal inflammation and loss of barrier function improved acute cholestatic liver injury and damped liver fibrosis in a chronic colitis model. This phenotype was independent of colitis-triggered alterations of microbial bile acid metabolism but mediated via hepatocellular NF-κB activation by lipopolysaccharide (LPS), which suppressed bile acid metabolism in-vitro and in-vivo. This study unravels a colitis-triggered protective circuit suppressing cholestatic liver disease and provides a strong rational for multi-organ treatment strategies for PSC.

L1.03 The gut microbiome controls liver regeneration through lipid metabolism

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DOI 10.1055/s-0042-1759893

Introduction The gut-liver axis has been implicated in liver disease and physiology although the underlying mechanisms remain unclear. We determined the functional contribution of the gut microbiome and its metabolites to liver regeneration in a preclinical mouse model and patient samples.

Methods Partial hepatectomy (PHx) was performed in C57Bl/6 mice after 3 days of antibiotic or control drinking water treatment. In addition, germ-free mice, minimally colonized mice (OMM12) and conventionally housed mice were subjected to PHx. Liver samples from mice were tested by qRT-PCR, immunoblot and immunohistochemistry. Intestinal contents and membrane lipid synthesis were analyzed by mass spectrometry. In vitro experiments were performed on human hepatoma cells and patient liver samples.

Results Antibiotic treatment induced colon dysbiosis in mice, accompanied by decreased hepatocyte proliferation, diminished liver regeneration capacity, and increased mortality after PHx. Mice treated with antibiotics had lower concentrations of short-chain fatty acids in the colon. In addition, expression of SCD1 (stearoyl-CoA-desaturase-1) was reduced in antibiotic-treated mouse liver after PHx. These results were independently confirmed in germ-free mice, but OMM12 mice essentially rescued hepatocyte proliferation and liver regeneration. A functional correlation between SCD1 expression and cell proliferation was also found in human hepatoma cells and in patient liver tissue.

Conclusion Intestinal dysbiosis caused by antibiotic treatment, affects the production of short-chain fatty acids, impedes lipid synthesis and regeneration of the liver. SCD1 could be a surrogate marker for liver regeneration in patients undergoing liver surgery.

Lecture Session II Clinical Hepatology, Surgery, LTX
27/01/2023, 15.10 pm – 15.55 pm, Lecture Hall

L2.01 The landscape of Autoimmune Hepatitis in Europe: first results of the prospective multicentre R-LIVER registry

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DOI 10.1055/s-0042-1759894

Background and Aims Prospective multicentre data on adult patients with autoimmune hepatitis (AIH) in Western countries is lacking. To address this, the European Reference Network for Hepatological Diseases (ERN RARE-LIVER) launched the prospective, quality-controlled R-LIVER registry on newly diagnosed people with AIH. Our aim was to assess therapeutic strategies and outcome in AIH patients across different European countries.

Methods Data was collected prospectively at time of initial diagnosis of AIH, after 6 and after 12 months follow-up. Patients with concomitant liver diseases other than fatty liver disease at diagnosis were excluded. Biochemical response was defined as normalization of transaminases.

Results 219 patients were included in the final analysis (81.3 % female) with a mean age at diagnosis of 53 years. Liver biopsy was performed in 95.4 %. 24 patients presented with acute-severe AIH, including three patients receiving liver transplantation. The latter were excluded for further analysis. After 6 months 51.4 % (111/216) and after 12 months, 63 % (136/216) patients showed biochemical response (26.5 % (57/215) of patients showed biochemical response after 12 months without steroids). At 1 year follow-up 32.1 % (69/215) of patients received 2nd/3rd-line treatment, mainly due to intolerance. Three patients died, two due to liver failure, one out of unknown reason.

Conclusion The results of this first European multicentre prospective AIH registry show that over one third of patients do not achieve normalization of transaminases after one year of treatment. The landscape of AIH-care is heterogeneous throughout Europe with high rates of failure of first line therapy.

L2.02 Extrahepatic Bile Duct Organoids as a Model to Study Ischemia/Reperfusion Injury during Liver Transplantation

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DOI 10.1055/s-0042-1759895

Introduction Biliary complications are still a major cause for morbidity and mortality after liver transplantation. Ischemia/reperfusions injury (IRI) leads to disruption of the biliary epithelium. Yet little is known about the underlying molecular mechanism. We introduce a novel model to study the effect of IRI on human cholangiocytes using extrahepatic cholangiocyte organoids (ECOs).

Method Extrahepatic bile duct tissue was collected during liver transplantation at static cold storage and after reperfusion (n = 15). Gallbladder tissue was used as control (n = 5). ECOs (n = 9) were cultured from extrahepatic biliary tissue. Multiplex immunofluorescence, in-situ hybridization and qRT-PCR were performed to study programmed cell death. IRI was induced in ECOs by introducing cells to a hypoxic chamber for 48h, followed by reoxygenation.

Results After reperfusion an activation of programmed cell death was observed ($p = 0.002$). Cultured ECOs formed circular structures recreating a tubular structure similar to that found in the bile duct. Analysis revealed a cholangiocyte phenotype of the ECOs. After hypoxia ECOs showed increased expression of ACSL4 ($p < 0.0001$) and VEGF- A ($p < 0.0001$). HIF1-α expression was increased after reoxygenation ($p < 0.0001$). Expression patterns were similar to those found in the bile duct biopsies at static cold storage.

Discussion ECOs are in-vitro cellular systems that self-organize through mechanisms like those found in-vivo. They recapitulate the structure and exhibit similar patterns of ACSL4, VEGF- A and HIF1-α expression as extrahepatic bile duct during liver transplantation and thus provide a suitable model to study IRI in choanocytes after liver transplantation.

L2.03 Long-term PPI treatment is a risk factor for acute-on-chronic liver failure in patients with advanced cirrhosis: a propensity score matched analysis

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DOI 10.1055/s-0042-1759896

Introduction Identification of risk factors for acute-on-chronic liver failure (ACLF) is of major importance, since ACLF is a fatal complication of cirrhosis. While a number of studies have reported an association of proton pump inhibitor (PPI) treatment with complications of cirrhosis , the impact of PPI treatment on the risk of ACLF is unclear. Therefore, the present study aimed to investigate if PPI treatment affects ACLF development in patients with cirrhosis.

Methods After retrospective screening of 642 patients undergoing first-time elective or emergency endoscopic band ligation of esophageal varices, 74 cirrhosis patients who received long-term PPI treatment following the intervention were 1:1 propensity score matched to 74 cirrhosis patients who did not receive PPI treatment. The included patients were followed-up for 3 years and the development of ACLF, mortality and upper gastrointestinal bleeding complications were recorded.

Results ACLF was significantly more frequent in the PPI group compared to the no-PPI group (50.0 % vs. 31.1 %, p = 0.032). ACLF-related deaths contributed significantly to an increased three-year mortality in the PPI group (55.4 % vs. 31.1 %, p = 0.025). Model for End-Stage Liver Disease (MELD) score and PPI treatment emerged as independent predictors of ACLF from multivariable Cox regression analyses. Of note, the impact of PPI treatment on the development of ACLF was strongest in patients with a MELD score > 12. Gastrointestinal bleeding events were not significantly reduced in the PPI group.

Conclusions Long-term PPI treatment is a risk factor for the development of ACLF

Lecture Session III Metabolism (incl. NAFLD) 27/01/2023, 17.50 pm – 18.35 pm, Lecture Hall

L3.01 Diurnal lipid and hepatic protein rhythms exhibit different population specific profiles

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DOI 10.1055/s-0042-1759897

Introduction Diurnal biological rhythms may have an influence on laboratory diagnostics and could affect assessment of disease state. However, the extent of variation due to biological rhythms is unknown for most parameters with clinical relevance.

Methods Datasets of over 500,000 subjects from hospital routine laboratory information systems were employed to develop canonical rhythms for measurands (albumin, total cholesterol) with known circadian oscillations. A Quantile Regression Model was applied for modelling hourly medians of serum levels (dependent variable) with time, age, and sex as independent variables. Time was transformed in a two-component cosinor function to ensure the cosinor rhythmometric.

Results From first values of patients' routine laboratory measurements from several German university hospitals, robust diurnal rhythmics covering the entire 24-hour period could be generated. These modelled rhythms agreed well with experimentally obtained data. Representative canonical rhythms of total cholesterol and albumin are depicted for 18-39 year old and ≥ 60 year old females. For cholesterol, a sex- and age-independent bimodal acrophase was identified. In contrast, we observed a stronger deformation of the albumin trajectory in females ≥ 60 years than in those of 18 – 39 years.

Conclusions Potential influence of biological rhythms on clinical laboratory parameters should not be established solely in young, healthy subjects but in an age-dependent manner, to capture the full potential bias associated with day-time dependent variations.

L3.02 In vivo adenine base editing reverts C282Y and improves iron metabolism in hemochromatosis mice

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DOI 10.1055/s-0042-1759898

Hereditary Hemochromatosis (HH) is one of the most common genetic diseases in the Caucasian population, with a prevalence of 1:200/400. Among the four different types, the most common form is Type 1, a homozygous p.C282Y variant in the HFE gene, in which a guanosine is replaced by an adenosine (c.845 G > A). This variant results in the misfolding of the HFE protein, which can no longer reach the cell membrane of the hepatocytes, thereby losing its ability to work as a sensor for the iron content in the bloodstream. This ultimately causes the accumulation of iron in various organs, mostly in the liver, heart and pancreas, thus leading to the development of chronic diseases. A major complication represents the development of hepatocellular carcinoma.

Here, we developed an innovative strategy to correct the G > A point mutation in the HFE gene in a permanent way, by using the adenine base editor ABE7.10. The generation of an in vitro system tailored to the HFE sequence allowed us to screen various sgRNAs. The best performing guide, together with the base editor, was then applied in 129-Hfe tm.1.1Nca mice using the AAV8 Split vector technology. Our treatment led to a base conversion rate in the HFE gene of > 10 % and it improved iron metabolism in the liver. This proof-of-concept study demonstrates the therapeutic potential of adenine base editor for HH.

L3.03 Exploring the effects of different bile acids receptor agonists in Non-Alcoholic Steatohepatitis (NASH) and NASH-related hepatocarcinogenesis

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Non-Alcoholic Fatty Liver Disease (NAFLD) is the hepatic manifestation of the metabolic syndrome and presents as a condition with progressive severity. Non-Alcoholic Steatohepatitis (NASH) consists in its most detrimental stage, preceding liver cirrhosis, and is an independent risk factor for developing

Hepatocellular Carcinoma (HCC), the most common primary liver cancer. NASH currently lacks approved treatments and further studies to develop new therapeutic strategies are urgently required. Several studies highlighted the importance of the bile acid receptor FXR in regulating glucose and lipid metabolism, making it an appealing target in the context of obesity, NASH and cholestatic disease. Currently, several FXR agonists are being tested in clinical trials for NASH. However, the efficacy of the compounds available at the moment seems to have a large spectrum of outcomes, and we were interested in identifying the best candidate and defining the features of its action. By employing dietary models of NASH in rodents, we tested different FXR agonists in preventive and therapeutic ways. The animal experiments were carried out in parallel with mainly histological analysis, classical molecular biology assays, flow cytometric measures and major Omics analysis performed on the tissue collected. Our results show that only one of the compounds tested was really able to effectively reverse the major features of NASH and metabolic syndrome, in particular steatosis and inflammation. However, some key findings revealed the activation of pro-carcinogenic pathways, which could potentially expose the liver to an additional load of signals that drive malignant transformation.

Lecture Session IV Tumors 28/01/2023, 09.05 am – 09.50 am, Lecture Hall

L4.01 Deep learning-enabled diagnosis of liver adenocarcinoma

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DOI 10.1055/s-0042-1759900

The diagnosis of a gland-forming carcinoma within the liver is a frequent scenario in routine pathology with critical impact on clinical decision-making. However, rendering the correct diagnosis can be challenging and often requires integration of clinical, radiological and immunohistochemical information. We present a deep learning model (HEPNET) to distinguish intrahepatic cholangiocarcinoma (iCCA) from colorectal liver metastasis (CRM) as the most frequent primary and secondary forms of liver adenocarcinoma at clinical-grade accuracy from hematoxylin and eosin-stained whole-slide images. HEPNET was trained on 714 589 image tiles from 456 patients randomly selected in a stratified manner from a pool of 571 patients that underwent surgical resection or biopsy at Heidelberg University Hospital. Model performance was evaluated on a hold-out internal test set comprising 115 patients and externally validated on 90 patients recruited at Mainz University Hospital. On the hold-out internal test set, HEPNET achieved an AUROC of 0.994 and an accuracy of 96.522 % on the patient-level. Validation on the external test set yielded an AUROC of 0.997, corresponding to an accuracy of 98.889 %. HEPNET significantly outperformed six pathologists with different levels of experience in a reader study on 50 patients, boosted the performance of resident pathologists to the level of senior pathologists and reduced potential downstream analyses. We here provide a ready-to-use tool with clinical-grade performance that may facilitate routine pathology in both rendering a definitive diagnosis and guiding ancillary testing. Incorporation of HEPNET into pathology laboratories may optimize diagnostic workflow complemented by test-related labor and cost savings.

L4.02 Elevated serum levels of CCL23 are associated with poor outcome after resection of biliary tract cancer

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DOI 10.1055/s-0042-1759901

Background Surgical resection is the only potentially curative treatment for patients with biliary tract cancer (BTC). However, 5-year survival rates are below 50 % mainly due to tumor recurrence. The preoperative identification of ideal surgical candidates has remained a major challenge. CCL23 has been associated with tumor progression in hepatocellular carcinoma (HCC) but its role in the context of BTC is largely unknown. Here, we evaluated circulating levels of CCL23 as potential diagnostic and prognostic biomarker in patients with resectable BTC.

Methods CCl23 serum levels were analyzed by multiplex immunoassay in a cohort of 119 BTC patients receiving tumor resection as well as 50 healthy controls and 11 patients with primary sclerosing cholangitis (PSC).

Results Baseline serum CCL23 levels were significantly elevated in BTC patients compared to PSC patients as well as healthy controls. CCL23 increased the diagnostic sensitivity and specificity of established tumor markers including CA19-9 and correlated with patients' age and makers of systemic inflammation. Elevated preoperative CCL23 levels were associated with a significantly impaired postoperative outcome. BTC patients with a preoperative CCL23 level above the optimal prognostic cut-off value of 702.4pg/ml showed a median OS of only 110 days compared to 501 days for patients with low initial CCL23 levels. The prognostic value of circulating CCL23 was confirmed in Cox-regression analysis.

Conclusion Serum levels of CCL23 are elevated in patients with BTC, and high preoperative CCL23 levels were associated with an impaired postoperative survival. CCL23 serum levels could help to identify the ideal surgical candidates for BTC resection.

L4.03 Changing treatment landscape associated with improved survival in advanced hepatocellular carcinoma: a nationwide, population-based study

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DOI 10.1055/s-0042-1759902

Background The treatment of hepatocellular carcinoma (HCC) is undergoing a historic transformation with the availability of several new systemic therapies. Sorafenib represented the only effective treatment option available for a long time. In the last few years, however, a number of new systemic therapies have been proven effective in large clinical trials and gained approval. The impact of

this changing landscape has not yet been studied in a German cohort in a nationwide, real-world setting.

Methods This observational, retrospective study is based on a claims data base representative for the German population. To investigate the effect of HCC systemic therapies in this dataset, we selected all HCC patients between 2015 and 2020 (ICD-10-GM C22.0). The study group was divided into two groups: Group A (2015–07/2018) consisted of patients receiving systemic therapy prior approval of Lenvatinib and, Group B (08/2018–2020) receiving new therapeutic options such as Lenvatinib. OS was estimated with Kaplan-Meier analysis, adjustment for demographics and comorbidities was performed. Annual treatment costs were calculated.

Results In the dataset, a total of 460 HCC patients received systemic therapy in 2015-2020. Based on the date of first drug administration, 255 patients received their treatment prior to approval of Lenvatinib (group A), and 205 afterwards (group B). Patient characteristics were distributed equally. Median overall survival was markedly increased in group B. Prolonged patient survival was associated with higher treatment costs.

Conclusions The introduction of multiple new treatment options resulted in substantial survival improvements of patients with aHCC in Germany.

Lecture Session V Viral Hepatitis and Immunology
28/01/2023, 11.45 am – 12.30 pm, Lecture Hall

L5.01 Infectivity of an ancient hepatitis B virus cloned after retrieval from archaeological human remains

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DOI 10.1055/s-0042-1759903

Background HBV evolution, diversity and phylogeographic history remain unclear. The successful retrieval of HBV DNA from archaeological human remains allows genotypic and phenotypic studies of HBV lineages over thousands of years. Aim of this study was to generate, clone and characterize the infectivity of a 1,160-years-old HBV isolate of genotype D (GT-D, DA222) in vitro and in vivo.

Methods The ancient HBV strain (DA222) was cloned based on shotgun sequencing from archaeological human remains (Mühlemann, Nature 2018). DA222-HBsAg-pseudotyped-(ps)HDV was used to infect HepG2-NTCP cells. HBV generated from 1.5mer plasmids was used to infect primary human hepatocytes (PHH) and humanized mice (USG). Viral markers were measured by RT-PCR, Abbott Architect system and immunohistochemistry.

Results Infection of HepG2-NTCP cells with DA222-HBsAg-psHDV corroborated the NTCP-conserved mode of infection, while genuine DA222 showed productive infection in PHHs. DA222 and a modern GT-D control displayed similar kinetics of HBV DNA viremia development (median 2E9 vs. 7E9, respectively; week 15) in humanized mice. pgRNA in serum (3E7 vs. 2E8 copies/ml) and liver (23 vs. 41 rel. exp.), as well as intrahepatic rcDNA (294 vs. 1479 copies/PHH) were slightly lower in DA222-infected mice, whereas cccDNA loads (1.5 vs. 2 copies/PHH) and human ISGs (MXA; ISG15; IP10; STAT1; OAS1) appeared comparable. Immunofluorescence indicated a typical HBcAg staining pattern.

Conclusion We established a productive infection of an ancient HBV strain. The in-vivo-replication capabilities of DA222 were nearly as good as modern HBV. Our approach opens new possibilities for phenotypic characterization of infection and pathogenic potential of ancient HBV lineages.

L5.02 Membrane-bound and soluble immune checkpoints are abundant in ascitic fluid from patients with decompensated cirrhosis

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DOI 10.1055/s-0042-1759904

Background Immune checkpoint receptors (ICRs) regulate immune responses by balancing the stimulatory and inhibitory signals delivered to immune cells by antigen-presenting cells. Evidence suggests that membrane-bound and soluble ICRs are dysregulated in liver disease, particularly in alcoholic hepatitis. We hypothesized that membrane-bound and soluble ICRs are compartmentalized and aberrantly regulated in patients with acute liver failure (ACLF) and spontaneous bacterial peritonitis (SBP).

Methods Paired ascitic fluid (AF) and blood samples from 80 patients with decompensated cirrhosis were analyzed for T cell-bound and soluble ICRs by flow cytometry, ELISA, and Luminex technology. Patients were stratified according to the presence of SBP (n = 30) or ACLF (n = 40).

Result Compared with serum levels, AF was a soluble ICR-rich compartment for all receptors measured, with sTIM-3 being the most abundant. Membrane-bound PD-1 and LAG-3 were significantly more abundantly expressed in peritoneal CD3 + T cells than in circulating T cells. SBP did not significantly affect the levels or expression of soluble or membrane-bound ICR, except for increased membrane-bound CTLA-4 expression during SBP in both blood and AF. Serum levels of sTIM-3 were increased during ACLF compared with acute decompensation without ACLF, whereas membrane-bound ICRs remained stable during ACLF. Serum levels of sTIM-3 and sLAG-3 correlated with liver dysfunction and systemic inflammation.

Conclusion Patients with end-stage liver disease have significantly higher ICRs in AD compared with blood, which may contribute to their systemic and local immune homeostasis. Targeting the balance between membrane-bound and soluble ICR in the peritoneal cavity could contribute to the control of SBP.

L5.03 Fate of HDV-specific CD8 + T cells during bulevirtide monotherapy in patients with chronic hepatitis delta

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DOI 10.1055/s-0042-1759905

Background and Aims Bulevirtide reduces HDV viremia in chronic HDV infection, however, long-term treatment is required to prevent relapse. Sustained treatment response may be fostered by therapy-induced restoration of HDV-specific CD8 + T cells that are exhausted during chronic HDV infection. We thus studied the effect of bulevirtide monotherapy on HDV-specific CD8 + T cell repertoire, phenotype, and functionality.

Methods PBMC and sera were collected from 33 HBV/HDV co-infected patients, including 19 cirrhotic patients starting bulevirtide treatment, up to 40 weeks on-treatment. HDV-specific CD8 + T cells were analyzed using overlapping L-HDAg peptides and optimal epitopes. Ex vivo high-dimensional flow cytometry analysis of multimer + CD8 + T cells was performed longitudinally in selected patients. HDV sequences were analyzed.

Results In two-thirds of patients, an HDV-specific CD8 + T cell response was detectable, but did not substantially increase during treatment. Importantly, > 2/3 of HDV-specific CD8 + T cell responses targeted viral epitopes with sequence variations consistent with viral escape mutations. Only the few HDV-specific CD8 + T cells targeting conserved epitopes displayed a terminally exhausted phenotype that shifted to a memory-like phenotype after suppression of HDV load.

Conclusion Antiviral treatment with bulevirtide is able to partially restore exhausted HDV-specific CD8 + T cells targeting conserved viral epitopes. Since the majority of HDV-specific CD8 + T cell responses target escaped viral epitopes, this effect does not translate to a substantial improvement of overall HDV-specific CD8 + T cell immunity. Our findings may explain in part that long-term bulevirtide treatment is required to achieve sustained viral clearance.

Poster Visit Session I Basic Hepatology (Fibrogenesis, NPC, Transport) 27/01/2023, 12.30 pm – 13.15 pm

P1.01 Hepatic angiocrine HGF weakens liver fibrogenesis by modulating the PDK1/Akt axis

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Introduction Hepatocyte growth factor (HGF) is a complete hepatic mitogen and is thought to play a role in liver fibrogenesis and hepatocarcinogenesis. Liver sinusoidal endothelial cells (LSEC) can recruit inflammatory cells by releasing angiocrine signals to produce HGF in the process of liver fibrosis and cirrhosis, but the precise contributions of HGF from LSEC to liver fibrosis remain elucidated.

Methods To study the effects of hepatic angiocrine HGF on liver fibrogenesis, Stab2-Cretg HGFfl/fl (HGFLSEC-KO) mice, in which HGF is specifically knocked out in LSEC, were used. These mice were repeatedly injected with carbon tetrachloride (CCl4) for six weeks. After onset of cirrhosis, we analyzed liver-to-body weight ratio kinetics, immunohistochemistry, Western blot and RT-PCR for fibrotic markers, HGF/c-MET-related pathway components and cell cycle-associated genes.

Results We found that HGF-LSEC-KO mice showed no difference in relative liver weights after early-stage CCl4 treatment. However, HGF-LSEC-KO mice exhibited higher expression levels of collagen 1A1 and alpha-SMA, and hepatocyte proliferation was significantly impaired in HGF-LSEC-KO mice. Furthermore, LSEC-specific HGF deficiency deactivated the PDK1/Akt pathway, while hepatic angiocrine HGF did not alter immune cell infiltration. Aquaporin-4 (AQP4) was identified to be highly expressed in HGF-deficient LSEC by RNA sequencing, which could be confirmed by consecutive expression analysis.

Conclusion The hepatic angiocrine HGF signaling pathway plays a crucial role in early stages of liver fibrogenesis and is essential not only for liver recovery during fibrogenesis, but also for liver and even whole organism growth.

P1.02 Hepatocyte specific knockout of c-Jun reduced liver damage in S. mansoni infected mice

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Introduction Schistosomiasis is a widespread parasitic disease caused by schistosome worms with about 230 million people affected worldwide. A Schistosoma mansoni couple produces about 300 eggs daily, which can lead to granuloma formation and liver fibrosis. The transcription factor c-Jun is involved in liver regeneration, proliferation, and apoptosis. We demonstrated previously that hepatocellular c-Jun is permanently induced by S. mansoni infection. Aim of our present study was to examine the function of c-Jun in hepatocytes of S. mansoni infected mice.

Methods 12 hepatocyte specific c-Jun knockout mice (Alb-Cre/loxP-System) and 12 c-Junf/f mice were infected at the age of 8 weeks in a water bath with 100 cercariae (♂ + ♀) of S. mansoni. Non-infected littermates served as controls and supercontrols (n = 6 in each group). Liver damage, histologic alterations and liver specific biomolecular alterations were analyzed by functional tests, western blotting and immunostaining in serum, liver tissue and histologic slices of the animals after 9 weeks of infection. Group-differences were analyzed by one-way ANOVA using SPSS26.0.

Results Hepatocellular deletion of c-Jun was demonstrated by c-Jun immunostaining and western blotting. Serum alanine transaminase (ALT) was increased in serum of infected animals with a higher increase in the knockout group. Results from western blots suggest that parasite-derived dysregulated hepatic levels of PCNA, MCM2, CyclinD1, and cleaved caspase-3 were ameliorated by c-Jun.

Conclusion Our findings imply that c-Jun has a protective effect on hepatocytes during S. mansoni infection. First results propose that this protective effect of c-Jun could be mediated by controlling cell cycle and apoptosis.

P1.03 Role of fibroblast growth factor 9 in the regulation of hepatic bile acid homeostasis

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Altered bile acid (BA) homeostasis fosters progression of chronic liver disease. Cholesterol 7α-hydroxylase (CYP7A1) and sterol 27-hydroxylase (CYP27A1) are rate-limiting enzymes of BA synthesis. Besides, hepatic transporters, including sodium-dependent taurocholate cotransporting polypeptide (NTCP) and members of organic anion transporting polypeptide (OATP) family, determine BA homeostasis. Fibroblast growth factor 19 (FGF19)-mediated downregulation of CYP7A1 is known to protect from cholestatic liver injury. However, the role of other FGF family members in BA homeostasis is unknown.

The aim of this study was to analyze the role of FGF9, a paracrine FGF secreted by activated hepatic stellate cells, in BA homeostasis.

Methods and Results Treatment with recombinant FGF9 reduced CYP7A1, CYP27A1, NTCP and OATP2B1 expression in murine liver organoids and human hepatoma cells. Mechanistically, FGF9 induced JNK signaling in human hepatoma cells. Experiments applying a specific JNK inhibitor (SP600125) revealed that FGF9-induced JNK activation was involved in transcriptional repression of CYP7A1, CYP27A1 and OATP2B1. Contrarily, FGF9 diminished NTCP mRNA expression independent of JNK activation. Repressive effects of FGF9 on expression of BA synthesis enzymes and BA uptake machinery were almost completely abrogated by the FGFR1/2/3 inhibitor BGJ398, while selective FGFR4 inhibitor BLU9931 had no significant effect.

Summary and conclusion Our data indicate FGF9 as novel paracrine regulator of hepatic BA homeostasis. FGF9 suppresses transcription of CYP7A1 and CYP27A1 gene via binding to FGFR1/2/3 and activation of JNK signaling. Furthermore, FGF9 represses BA uptake via inhibition of NTCP and OATP2B1 expression. Herewith, FGF9 might protect from BA overload in chronic liver disease.

P1.04 Hepatozelluläre Proliferationssteigerung durch Inaktivierung des Transkriptionsfaktor FOXO3a bei Infektion mit Schistosoma mansoni

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Fragestellung Schistosomiasis ist eine parasitäre Infektionskrankheit, die von der WHO als neglected tropical desease eingestuft wird und mit schwerwiegenden klinischen Symptomen einhergeht. Die Eier des Parasiten Schistosoma mansoni gelangen in die Leber und führen dort zu einer Granulombildung. Der Transkriptionsfaktor FOXO3a reguliert in der Leber u.a. den Zellzyklus, mittels Verminderung der Zellteilung. Das Ziel unserer Arbeit war die Untersuchung der hepatischen Regulation, Aktivität und Lokalisation von FOXO3a bei Infektion mit S. mansoni.

Methoden Untersucht wurde Lebergewebe chronisch infizierter Hamster mittels Western Blot. Als Kontrollen dienten Lebern einer nicht infizierten Gruppe und Lebergewebe von Hamstern, die nur mit Zerkarien eines Geschlechts infiziert wurden. Zu den jeweiligen Gruppen wurden subzelluläre Fraktionierungen durchgeführt und mittels Western Blot untersucht. Für in vitro-Experimente wurden HepG2-Zellen mit S. mansoni-Antigenen stimuliert und die FOXO3a Expression mittels siRNA und Überexpression moduliert, wodurch die Untersuchung der Expression von Zellzyklusmarkern (CyclinB1, SKP2) in Abhängigkeit von FOXO3a ermöglicht wurde.

Ergebnisse Im Western Blot zeigte sich eine gesteigerte Expression von FOXO3a in Lebern infizierter Hamster im Vergleich zu den Kontrollgruppen, wobei eine positive Korrelation mit der Eilast bestand. In den nukleären Extrakten hingegen fanden wir vermehrt FOXO3a im Zellkern der Hepatozyten nicht-infizierter Tiere. Die mechanistischen Zellkulturexperimente geben erste Hinweise auf eine FOXO3a-vermittelte Aktivierung des Zellzyklus durch S. mansoni.

Schlussfolgerung Die Ergebnisse zeigen, dass bei einer Infektion mit S. mansoni FOXO3a aus dem Zellkern der Hepatozyten in das Zytoplasma transportiert wird, was zur Inaktivierung des Transkriptionsfaktors führt. Erste mechanistische Untersuchungen weisen auf eine hepatozelluläre Proliferationssteigerung durch S. mansoni mittels Inaktivierung von FOXO3a hin.

P1.05 Schistosoma mansoni eggs can be destroyed by Myeloperoxidase (MPO)-producing cells

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Introduction Schistosomiasis is one of the most prevalent parasitic diseases worldwide, with over 200 million affected people. Schistosome parasites that live in the mesenteric veins produce eggs, which can be trapped in the liver causing inflammatory responses and liver fibrosis. Hepatic eggs can be removed by immune cells months after successful praziquantel therapy. Neither precise cellular mechanisms nor molecular processes of this clearing action have yet been elucidated.

Methods Male C57BL/6 mice were infected with 100 cercariae each of the species Schistosoma mansoni. Histologic grading, qRT-PCRs, immunohistochemistry, and Proteome Profiler array analyses were conducted to determine hepatic damage and inflammation. S. mansoni eggs were cultivated in vitro with active and inactivated myeloperoxidase (MPO) for 48 hours and afterwards stained with Calcein, Hoechst, and SytoxOrange to determine their vitality.

Results S. mansoni induced a typical hepatic granulomatous inflammation in infected mice. About 20 % of the eggs, however, appeared disrupted. Remnants of these eggs were surrounded and mostly filled by CD11b + MPO-expressing cells. Interleukin-6- and TNF-α-levels, both Th1-cytokines, were increased by infection. Proteome Profiler analysis indicated an increased amount of MPO in the liver tissue of infected mice. In vitro cultivation of S. mansoni eggs with MPO reduced their viability compared to the control eggs cultivated with heat inactivated MPO.

Conclusion Our results suggest that neutrophil granulocyte derived MPO is involved in devitalizing and removal of S. mansoni eggs from the mouse liver.

P1.06 A common variant in the hepatobiliary phospholipid transporter modulates liver injury in PBC but not in PSC

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Background Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) represent two chronic cholestatic liver diseases. The ABCB4 gene encodes the hepatobiliary phospholipid transporter and its genetic defects cause cholestatic phenotypes (Stättermayer et al., J Hepatol. 2020). Here we investigate the ABCB4 c.711A-T genetic variant in patients with PBC and PSC.

Methods Two cohorts of patients were analysed. The Szczecin cohort comprised 196 patients with PBC (174 females, 42 % with cirrhosis) and 135 patients with PSC (39 females, 39 % with cirrhosis). The Warsaw cohort consisted of 260 patients with PBC (241 females, 44 % with cirrhosis) and 276 patients with PSC (97 females, 33 % with cirrhosis). The control cohort was composed of 468 individuals. ABCB4 c.711A-T was genotyped using TaqMan assay.

Results In both PBC cohorts, carriers of the risk ABCB4 c.711A-T variant presented more frequently with cirrhosis (Szczecin: OR = 1.841, P = 0.025; Warsaw: OR = 1.528, P = 0.039). The association between ABCB4 c.711A-T and cirrhosis risk in PBC remained significant (P = 0.03) in multivariate regression models. The ABCB4 risk allele was also associated with increased serum AST, GGT and ALP. During 8 ± 4 years follow-up, a total of 22 patients in the Szczecin PBC cohort developed cirrhosis and this risk was higher among carriers of the risk ABCB4 variant (OR = 5.65, P = 0.04). In contrast to PBC, the ABCB4 polymorphism had no effects on liver phenotypes the PSC.

Conclusions The frequent ABCB4 c.711A-T polymorphism modulates liver injury in PBC. Its inclusion in the diagnostic work-up of PBC patients might help to quantify their risk of progressive

P1.07 Bacterial translocation in patients with liver cirrhosis is promoted by bacteria-mediated mechanisms targeting essential functions of the epithelial barrier

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Background Spontaneous bacterial peritonitis (SBP) is a severe complication of liver cirrhosis. A crucial event in SBP development is bacterial translocation from the intestine to mesenteric lymph nodes. We characterized the interplay of intestinal epithelial cells with SBP-inducing bacteria and focussed on effects on cell-cell-contacts and cellular stress responses.

Method Mucus layer and protein levels of E-cadherin, occludin, p53 and p73 were examined in colonic biopsies from patients with liver cirrhosis and control patients who underwent screening colonoscopy. In vitro, Caco-2 and HCT-116 cells were co-cultured with Escherichia coli (E. coli; ATCC25922, O6:Hnt or patient-derived E. coli). Protein regulation of E-cadherin, occludin, p53 and p73 was evaluated by Western Blot. Cell cycle regulation and cell death were investigated by flow cytometry.

Results Patients with liver cirrhosis display a reduced mucus thickness, diminished levels of E-cadherin and occludin and markedly reduced levels of p53 and p73 in their colonic mucosa. In vitro, direct contact of Caco-2 or HCT-116 cells with E. coli induced degradation of E-cadherin and occludin, cell cycle arrest and enhanced epithelial cell death by a non-apoptotic mechanism. Upon co-cultivation both cell lines initially upregulated p53 and p73, whereas a reduction of these proteins was observed thereafter. This mechanism was dependent on MDM2 and coincided with cell death induction.

Conclusion Impaired epithelial integrity facilitates bacterial translocation in patients with liver cirrhosis. Active downregulation of cell junction components and p53 proteins by SBP-inducing bacteria suggests a bacteria-mediated mechanism targeting essential functions of the intestinal epithelium to drive development of SBP.

P1.08 MicroRNA-ITGA6/ Has2 signaling regulates liver fibrosis

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Liver fibrosis and cirrhosis are chronic liver diseases which contribute to the deaths of millions of individuals annually and together represent a major healthcare burden, globally. On the cellular level, the activation of quiescent hepatic stellate cells (HSCs) into pro-fibrotic myofibroblasts is one of the key drivers of fibrogenesis. HSCs change their transcriptional and epigenetic signature, and change their microRNA (miRNA) expression pattern. However, identification and in vivo functional analyses of miRNAs, which can modulate pro-fibrotic myofibroblasts remain to be investigated. We aimed to identify miRNAs that can suppress the activation of myofibroblasts in vitro and to analyze anti-fibrotic potential of the identified miRNAs in vivo.

Based on multiple miRNA screens in primary human myofibroblasts, we identified two miRNAs, as suppressors of the pro-fibrogenic profile of myofibroblasts in vitro. Further, we overexpressed these two miRNAs via adeno-associated viral vectors in various murine models of liver fibrosis. Overexpression of these miRNAs ameliorated periportal and pericentral liver fibrosis. To elucidate the molecular mechanisms, we performed microarray analyses. As a result, hyaluronan synthase 2 (HAS2) and integrin alpha-6 (ITGA6) were discovered as the targets of the identified miRNAs.

Taken together, our findings provide evidence that miRNA modulation in myofibroblasts presents a promising approach for the treatment of liver fibrosis. These findings open new avenues for the development of novel therapeutics, that employ miRNAs alone, or in combination with other drugs currently in clinical trials, for the treatment of liver diseases.

P1.09 Die Abcg5/g8-Defizienz in Leber oder Dünndarm führt zu vergleichbaren Phänotypen

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Hintergrund Der Transport von Cholesterin aus der Leber in die Galle erfolgt über den heterodimeren Transporter Abcg5/g8. Neben der Leber wird dieser Transporter auch im Dünndarm exprimiert, wo er den Cholesterintransport aus dem Enterozyten in das Darmlumen steuert. Ziel dieser Studie war der Vergleich von Phänotypen bei Fehlen des Transporters in Leber oder Darm.

Methodik Mithilfe der BAC-basierten Rekombination wurden leber- bzw. dünndarm-spezifische Abcg5/g8-defiziente Mäuse generiert (HepKO und IntKO). Nach der Kanülierung des Gallengangs wurde die hepatische Galle gesammelt und biliäre Lipide wurden quantifiziert. Außerdem wurden Genexpressionsanalysen Cholesterinstoffwechsel-relevanter Gene durchgeführt.

Ergebnisse IntKO-Tiere zeigten ein höheres Leber-/Körpergewicht-Verhältnis, wohingegen Leber- oder Körpergewicht allein sich nicht unterschieden. In der Galle der IntKO-Tiere waren die Gallensäuren erhöht und die Phospholipide erniedrigt, Cholesterin, Gesamtlipide und der Cholesterin-Sättigungsindex unterschieden sich nicht. Die Expression des alternativen Cholesterin-Transporters Abcg1, des Gallensäurentransporters Abcb11, sowie Mboat7 waren in den Lebern der IntKO-Tiere im Vergleich zu HepKO erniedrigt, während die Expression von Fxr, Npc1l1 und Srebf1 erhöht war. Alle anderen untersuchten Gene waren vergleichbar exprimiert. Die Expression von Abcg1, Hmgcr, Srebp2 und Ldlr war in den Därmen (Duodenum) der IntKO-Tiere im Vergleich zu HepKO erniedrigt, während die Expression Abcba1, Npc1l1, Slc10a2, Srebf1, Fxr, Mboat7 und Tm6sf2 nicht verändert war.

Schlussfolgerungen Die Ergebnisse zeigen, dass die gewebsspezifische Deletion des Cholesterintransporters in Leber und Darm hinsichtlich der Cholesterinexkretion und der Expression Cholesterinstoffwechsel-relevanter Gene zu vergleichbaren Phänotypen führt. Dies zeigt die Relevanz des Transporters für beide Organe.

P1.10 Immortalized human urine-derived cells for analysis of pathomechanisms in monogenic liver diseases

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For monogenic diseases, patient-derived cells are the gold standard of experimental studies. However, materials derived from biopsies are limited and the proliferation of primary cells is frequently restricted. Epithelial cells found in the urine (UCs) represent a noninvasive source for establishment of continuously growing cell lines. The aim of this study was to recapitulate pathomechanisms of various liver diseases using immortalized UCs via gene transfer of human papillomavirus 16-derived oncogenes E6 and E7 (HPVE6E7).

Urine cells were collected from patients having Wilson disease (WD), hereditary transthyretin amyloidosis (hATTR), HFE-related hemochromatosis, or autosomal dominant polycystic liver disease (ADPLD). After retroviral transduction of UCs by HPVE6E7, the derived cells indicated stable proliferation rates for more than one year without signs of senescence as assessed by cell proliferation assays, while primary UCs stopped proliferation after a few weeks. Sequence analysis indicated that the transgene was inserted into the chromosome. The immortalized UCs were positive for CD29, CD44 and CD13. CD71 was less prominently expressed, whereas CD105 and CD166 were undetectable. Analysis of mRNAs encoding epithelial (KRT7, OCLN and CLDN1), fibroblast (FN1 and VIM) and renal markers (SNAI2, L1CAM and SLC2A1) indicated a high expression compared to primary UCs. Moreover, ADPLD-derived immortalized cells encoding a novel Sec61A1 mutation showed a significant downregulation of markers regulating autophagy.

In summary, our data suggest that transgenic HPVE6E7 expression can technically overcome senescence in primary cells derived from liver disease patients allowing in vitro studies of disease-causing molecular pathways in the context of the patient-specific genetic background.

P1.11 Augmenter of liver regeneration (ALR) activates cytoplasmic β-Catenin by GSK-3β independent mechanisms

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Liver regeneration is orchestrated by a variety of factors amongst them ß-Catenin and Augmenter of Liver Regeneration (ALR), the latter is known for its anti-oxidative, anti-apoptotic and anti-inflammatory properties. ß-Catenin acts as a transduction molecule within the Wnt signaling pathway and is part of a membrane bound E-cadherin-protein complex, released upon EGF receptor activation. In the absence of a Wnt/Wingless signal, cytoplasmic ß-catenin interacts with GSK-3ß in the degradation complex, which competes with E-cadherin for binding to ß-catenin. Previously we have shown that ALR activates the EGF-receptor-PI3/Akt signaling pathway and thereby inhibiting GSK-3ß. Therefore we aimed to analyze the impact of ALR (exogenous and endogenous) on cellular ß-catenin signaling. We performed in vitro studies treating cell lines (Hep3B, Huh7), w/o stably expressing short form ALR (sfALR), with specific inhibitors and/or recombinant ALR (rALR) followed by western blotting. Treatment with rALR phosphorylates PI3/Akt and GSK-3ß, but did not change ß-catenin degradation or activation, detected by specific antibodies against ß-catenin phosphorylation at S33/S37/T41 or S552. Interestingly, cells expressing sfALR revealed increased total and reduced phosphorylated ß-catenin. On the other hand, rALR treatment enhanced phosphorylation of ß-catenin (Y654) even more than EGF compared to control cells. As previously shown for EGF treatment, phospho-(Y654)-ß-catenin (Y654) dissociates from E-cadherin, translocation to the nucleus, and increase transactivation by GSK-3ß independent mechanisms. Contrary to this potential mitogenic mechanism we found increased ß-catenin in sfALR expressing cells in agreement with our previous report of increased E-cadherin and ZO-1 expression in these cells and their anti-EMT properties.

P1.12 Loss of the mechanistic target of rapamycin complexes 1 (mTORC1) causes a lethal alpha-1 antitrypsin deficiency associated liver disease

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Introduction Alpha1-antitrypsin (AAT) mutations lead to the retention of the otherwise secreted protein in the endoplasmic reticulum (ER) thereby giving rise to AAT deficiency (AATD). Liver disease arising due to the proteotoxic stress is the second leading cause of mortality in AATD.

Aims & methodology Since mTOR signaling is a key coordinator of proteostasis, we assessed its importance in PiZ mice overexpressing the characteristic AAT mutation. They were cross-bred with rodents harboring a hepatocyte specific-ablation of the interaction partners Raptor or Rictor corresponding to mTOR complexes 1 or 2 (mTORC1/2) or with mice lacking mTOR.

Results At two month of age, PiZ-mTORΔhep and PiZ-RaptorΔhep but not PiZ-RictorΔhep mice showed signs of increased liver injury and apoptosis despite diminished AAT accumulation. While PiZ-RaptorΔhep animals displayed increased levels of the pro-apoptotic protein CHOP, CHOP ablation did not rescue the phenotype. PiZ RaptorΔhep mice started dying at 10 weeks of age, but serum proteomics revealed no signs of altered protein synthesis. Notably, PiZ RaptorΔhep mice harbored alterations in liver zonation with diminished glutamine synthetase (GS) levels. Additionally, enlargement of hepatocellular nuclei was accompanied by a downregulation of the proliferation regulators epidermal growth factor receptor (EGFR) and hepatocyte growth factor receptor (cMet). Liver proteomics demonstrated a reduction of the urea cycle protein ornithine carbamoyltransferase (OTC) that was confirmed by immunoblotting. Preliminary analyses revealed strongly increased serum ammonia as the likely cause of death.

Conclusions In Pi * Z mice, hepatocellular mTORC1 ablation seems to cause a lethal impairment of ammonia detoxification.

P1.13 EGF/STAT1-maintained ECM1 expression in hepatic homeostasis is disrupted by IFNγ/NRF2 in chronic liver diseases

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In healthy liver, L-TGF-β is stored in the extracellular matrix and stabilized in the inactive form by ECM1. Upon damage, ECM1 production is downregulated, especially in hepatocytes, leading to L-TGF-β activation and fibrogenesis. We used in silico promoter and ligand receptor interaction analyses with in vitro studies with mouse and human hepatocytes, to investigate the physiological regulation of ECM1 expression, and the pathological pathway mediating its downregulation in diseased liver.

ECM1 expression in hepatocytes is maintained by the EGF/EGFR/STAT1 pathway. Blocking the EGFR with Erlotinib inhibits ECM1 expression and STAT1 phosphorylation at S727 in hepatocytes and in mouse liver. Further, depleting Stat1 is sufficient to phenocopy EGFR inhibition, whereas targeting of Fos/Jun and cMyc has no effect, suggesting STAT1 as downstream mediator of the EGFR signal to maintain ECM1 expression. STAT1 binding to the Ecm1 gene promoter is confirmed by ChIP assays. Injury-mediated inflammation is providing high levels of IFNγ, which intercepts EGF signaling through downregulating EGFR, leading to the loss of ECM1 expression. Importantly, IFNγ induces STAT1 phosphorylation on Y701 position, which blunts the ability of S727-phosphorylated STAT1 to bind the Ecm1 gene promoter. Additionally, IFNγ induces NRF2 nuclear translocation, which directly binds to and negatively regulates the Ecm1 gene promoter, further reducing ECM1 expression, and therewith facilitating L-TGF-β activation and fibrogenesis. Administration of IFNγ to mice confirms the in vitro findings.

Our findings delineate the control of ECM1 expression in hepatocytes, which has potential for anti-fibrotic therapy development.

P1.14 MK2 limits the production of distinct acute-phase proteins by controlling the composition of gene expression modulators

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When faced with a pathogenic challenge, the liver displays a strong innate immune response by producing many acute-phase proteins (APPs). APPs counteract tissue damage and regulate repair processes. They also isolate and neutralize invading pathogens and prevent further pathogen entry. The expression of APPs by hepatocytes is induced by inflammatory cytokines, which are mainly released by macrophages. Many of the molecular mediators involved here are controlled by the protein kinase MK2. However, to date it is unknown, if MK2 plays a role for hepatic APP synthesis. Therefore, the aim of this study is to reveal MK2-dependent mechanisms involved in the regulation of APP synthesis in the liver.

Upon treatment with LPS, we observed that synthesis of many APP-inducing cytokines collapses in MK2-deficient mice when compared to wildtype. Surpri-

singly, transcript expression of only one APP target gene, namely α2-macroglobulin was abolished. Moreover, various APPs - including hepcidin and IL-1 receptor antagonist - were even more enhanced. Further data revealed that this was mediated by IL-6 and IL-1β and occurred on the level of transcription. Our genome-wide study uncovered a pattern of MK2-dependent modulators of gene expression that ultimately limit production of APPs like hepcidin. Conclusively, the kinase MK2 is essential for the production of many APP-inducing cytokines in the context of an inflammatory response of the organism, yet MK2 is only important for certain APPs. Most of these APPs are limited, rather than induced, in their expression due to an MK2-dependent assembly of a specific pattern of transcription factors.

P1.15 Spatio-temporal mathematical model describing the interplay between biomechanics and cell kinetics during fibrotic scar formation

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Liver fibrosis is characterized by the accumulation of overexpressed extracellular matrix (ECM) proteins as a result of exposure of tissue to repeated damage. There are distinct patterns of fibrosis such as collagen septa (from tissue sections called “fibrotic walls”) connecting two central veins due to toxic injury. In the past decade, some computational models using either rule-based models 2D or partial differential equations of liver fibrosis to study the cellular and molecular mechanisms. Within a 3D single-cell-based model resolving tissue microarchitecture, we now incorporate the collagen fiber mechanics to address fibrosis formation. The same model approach already simulated regeneration after acute liver damage hence fibrosis formation is a further step towards a digital liver twin. The pattern-characterizing parameters in this study were obtained through image analysis of images from animal experiments that were compared to human histopathology. We explored alternative model mechanisms and parameters for a detailed in silico study of possible mechanism on the formation of characteristic fibrotic walls in liver fibrosis.

P1.16 Retinoic acid generates a beneficial microenvironment for liver progenitor cell activation in acute liver failure

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Massive hepatic necrosis is the most severe lesion in acute liver failure (ALF). To survive, liver progenitor cell (LPC)-derived regeneration is required, which is initiated by the ductular reaction. In ALF, high levels of retinoic acid (RA) are secreted into the microenvironment by the activated hepatic stellate cells (HSCs), which is the active form of vitamin A that activates the retinoic acid receptor (RAR). In this study, we investigated the function of RA in LPCs through RNA sequencing analysis and functional assays in cells and patients. Our results showed that in the ALF condition with inflammation or during the cell culture in vitro, HSCs, including phHSCs and LX-2 cells, will be activated. Subsequently, activated HSCs secrete high levels of RA, inducing RARα nuclear translocation in LPC. Based on RNA-seq analysis and investigations in HepaRG cells, atRA treatment activates the WNT-β-Catenin pathway, enhances stemness genes expression, promotes energy storage, and elevates the expression of ATP-binding cassette (ABC) transporters partially dependent on RARα in HepaRG cells, which is pivotal for the LPC activation, expansion, and the subsequent differentiation into hepatocytes in the conditions of ALF. Further, these signaling pathways induced by atRA treatment were confirmed by co-culture HepaRG with LX-2 cells. In addition, ALF patients with positive RARα nuclear staining in the LPCs displayed a better MELD score compared to the ALF patients who lost RARα nuclear expression. In conclusion, RA secreted by activated HSCs promotes LPCs activation, which is significant for the subsequent LPC-mediated liver regeneration in ALF.

P1.17 Efficient and precise gene correction of Wilson disease H1069Q mutation in an iPS cell model using CRISPR/Cas9 genome engineering

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Clustered regularly interspaced short palindromic repeats (CRISPR) associated nuclease 9 (Cas9) technology has the potential to efficiently correct genetic diseases. Wilson disease (WD) is caused by an autosomal recessive gene defect in the copper transporting protein ATPase7B that leads to a cytotoxic copper concentration, prominently in the liver. The point mutation H1069Q is the most frequent mutation in the Caucasian population and was targeted here by using CRISPR/Cas9 in WD specific induced pluripotent stem cells (iPSCs). We asked whether a gene correction is feasible, safe and efficient. Moreover, we studied whether gene-corrected iPSCs maintain the ability to differentiate into hepatocyte-like cells (iHeps) and whether such cells escape from toxic copper.
Epithelial cells from freshly donated urine obtained from a WD patient carrying the compound heterozygous mutation H1069Q/N1270S were collected and reprogrammed into iPSCs. WD iPSCs were transfected with the plasmid PX459. H1069Q plus single-stranded oligo DNA nucleotides (ssODNs) for homology-directed repair (HDR). Single iPSCs clones were analyzed by Sanger sequencing followed by hepatic differentiation and MTT assays.
After genome engineering, 46 % of the cell clones indicated a gene correction of the H1069Q mutation. The second mutation N1270S was not affected indicating the high specificity of the methodology. Corrected iPSCs could be differentiated to iHeps and indicated an improved resistance to high copper concentrations.
The current study demonstrates that in vitro genome engineering with CRISPR/Cas9 has a remarkable therapeutic potential to cure WD, thus further contributing to novel therapeutic approaches for WD specifically and monogenetic rare diseases in general.

P1.18 Pioneer factor FOXA2 is essential for maintaining the urea cycle in acute liver failure

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DOI 10.1055/s-0042-1759923

Background and Aims Disruption of the urea cycle results in hyperammonemia and thus causes hepatic encephalopathy (HE), a lethal complication of acute liver failure (ALF). A complete urea cycle requires six enzymes, including the rate-limiting enzyme carbamoyl phosphate synthetase I (CPS1). To date, the detailed regulation of CPS1 transcription in order to maintain urea cycle in ALF remains largely unknown.
Methods Expression of CPS1 and transcription factors such as FOXA2 and C/EBPα was examined by immunohistochemistry in liver tissues collected from 78 ALF patients including 27 with HE. The regulatory mechanisms of these factors on CPS1 and OTC transcription were investigated in vitro. The effect of FOXA2 in ALF was further investigated in acetaminophen-treated mice with or without adeno-associated virus serotype 8 (AAV8)-Foxa2 injection.

Results Physiologically, CPS1 transcription requires FOXA2 to maintain chromatin accessibility on their enhancers, which provides open binding sites for C/EBPα. In ALF, hepatic C/EBPα expression is inhibited by inflammation. In this setting, retinoic acid receptor synergizes with FOXA2 to maintain CPS1 transcription. Once ALF patients suffer from massive hepatic necrosis, liver progenitor cells perform the urea cycle to prevent hyperammonemia by initiating a transcription network comprising FOXA2 and C/EBPα. In ALF, HE occurs in patients lacking expression of these transcription factors. In mice with acetaminophen-induced ALF, injection of Foxa2-AAV8 maintains urea cycle and prevents hyperammonemia.

Conclusions FOXA2 is essential for maintaining the urea cycle. Pharmaceutical induction of hepatic FOXA2 expression might represent a novel approach to treat HE in ALF.

P1.19 Extracellular Matrix Protein 1 (ECM1) attenuates hepatic fibrosis by inhibiting protease-mediated latent TGF-β (LTGF-β) activation

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DOI 10.1055/s-0042-1759924

Objective ECM1-KO results in deleterious hepatic fibrosis and excessive LTGF-β activation. We aim to uncover the mechanisms underlying both.

Design Functional assays were performed in immortalised and primary hepatic stellate cells(HSCs), WT or ECM1-KO mice, and healthy or diseased human liver samples.

Results DESeq showed expression increases of Thrombospondins, matrix-metalloproteases , ADAMTS proteases, and TGF-β target genes in ECM1-KO versus WT mice. Recombinant human ECM1 prevented TSP-1-/ADAMTS1-/MMP-2- and MMP-9-mediated LTGF-β activation and decreased the level of active TGF-β detectable by TGF-β reporter assay in conditioned supernatant from LX-2 and primary human HSCs as well as reduced expression of HSC-specific hepatic fibrosis markers on RNA and protein level. Similar findings were obtained when human ECM1 was overexpressed in LX-2 HSCs. Based on co-immunoprecipitation analyses, ECM1 associated with all four LTGF-β-activating proteases and preferentially bound to the active states of MMP-2 and MMP-9. Mechanistical studies revealed that ECM1 abrogates KRFK- and KTFR peptide-mediated LTGF-β activation, which represent the respective LTGF-β-activating aa-sequences in TSP-1- and ADAMTS1. Furthermore, ECM1 reduced the proteolytic activity of MMP-2 and -9 as assessed via MMP activity assay. In humans suffering from fibrotic and cirrhotic CLD, the expression of ECM1 decreased with progressing fibrosis whereas that of the LTGF-β activators TSP-1, ADAMTS1, MMP-2, and MMP-9 increased.

Conclusion ECM1 exerts its hepatoprotective effect via inhibition of protease-mediated LTGF-β activation either through interacting with the specific LTGF-β-activating motifs or reducing the proteolytic activity directly, hence attenuating TGF-β-induced fibrosis and could thus serve as a template for potential future anti-fibrotic therapies.

P1.20 Repeated toxic injuries of murine liver are tolerated through minute steatosis and mild inflammation

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DOI 10.1055/s-0042-1759925

The liver has a remarkable capacity to regenerate and thus compensates for repeated injuries through toxic chemicals, drugs, alcohol, or malnutrition for decades. However, how and when tolerable damaging insults alternate the liver is still largely unknown. Over ten weeks, we induced repeated liver injuries in a mouse model by injecting carbon tetrachloride (CCl4) twice a week. We lost 10 % of the study animals within the first six weeks, which was accompanied by a steady deposition of extracellular matrix (ECM) regardless of the metabolic activity of the liver. From week six onwards, all mice survived, and in these mice, ECM deposition was rather reduced, suggesting ECM remodeling as a liver response contributes to better coping with repeated injuries. The data of time-resolved paired transcriptome and proteome profiling of 18 mice were subjected to multi-level network inference, using Knowledge guided Multi-Omics Network inference (KiMONo), which identified multi-level key markers exclusively associated with the injury-tolerant liver response. Interestingly, cancer and inflammation pathways were upregulated and validated using independent data sets compiled of 1034 samples from publicly available human cohorts. A yet undescribed link to lipid metabolism in this damage-tolerant phase was identified and confirmed by immunostaining of lipid droplets (steatosis). We identified week six as a critical switching point in the liver response program from an acute response that fosters ECM accumulation, to a tolerant "survival" phase with pronounced deposition of small lipid droplets that potentially protect against repetitive injury with toxic chemicals.

P1.21 Monitoring TGF-β1 effects on liver cells in vivo

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DOI 10.1055/s-0042-1759926

Transforming growth factor (TGF-β) plays an important role in the progression of chronic liver diseases by influencing a plethora of cellular processes, such as hepatic stellate cell activation and matrix remodeling, proliferation control or modulating the immunological niche. In order to test direct and chronic effects of TGF-β1, we treated healthy C57BL6/J mice with recombinant (r)TGF-β1 (100µg/kg, iv) for (2 and 24h) and injected the mice intravenously with an AAV8-TGF-β1 construct (10x1010 virus particles). AAV8-YFP (10x1010 virus particle) served as control. rTGF-β1 treated and part of the AAV8 treated mice were used for cell isolation and comparative scRNASeq analyses. Chronically affected mice were additionally used for a thorough morphopathological analysis. Surprisingly, AAV8-TGF-β1 infected mice survived only for 7 days. Plasma of AAV8-TGF-β1 infected mice displays significantly elevated TGF-β1 levels and the liver tissue shows strongly induced Smad phosphorylation, mainly in non-parenchymal cells, since mouse hepatocytes already display intrinsic nuclear

pSmad staining. Histopathological investigation of the livers reveals activation of hepatic stellate cells, upregulation of laminin expression and development of a basal membrane in perivenous and sinusoidal compartments, disturbance in zonation with loss of approximately one-third of glutamine synthetase (GLUL) expressing hepatocytes, and gain of additional E-cadherin positive cells. Further, loss of LYVE1 (fenestration marker) and increase in CD34 indicate capillarization. We currently investigating mice exposed to a lower dose of AAV8-TGF-β1 (1x1010 virus particle).We conclude that chronic challenging of liver cells with active TGF-β1 in healthy liver is associated with sinusoid capillarization, HSC activation and perivenous and sinusoidal scarring.

P1.22 KEAP1 deletion rescues cell death associated with GPX4 knockdown in hepatocytes in acute models of liver damage.

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DOI 10.1055/s-0042-1759927

Question Hepatocyte death is a process common to almost all liver diseases and is widely used to diagnose acute liver injury (ALI). Recently, the role of ferroptosis in carbon tetrachloride (CCl4)-induced ALI in mice has been suggested. This process is initiated by iron accumulation, the generation of reactive oxygen species (ROS), and subsequent lipid peroxidation leading to membrane damage and cell death. The inactivation of cellular antioxidant mechanisms, such as the glutathione-glutathione peroxidase 4 (GPX4) system, is recognized as a hallmark of ferroptosis. In this work, we aim to study the involvement of ROS in ALI in a model of ferroptosis.

Methods We generated mouse lines with hepatocyte-specific deletion of Gpx4 (GPX4-hepa) and Keap1 (GPX4/KEAP1-hepa), a key gene in ROS response, to evaluate ALI. We employed CCl4 and bile duct ligation acute models, both 48 hours of time.

Results Deletion of Gpx4 causes extensive liver damage that is associated with higher serum transaminase levels and an increased macrophage population in the liver during acute injury. Histological analyses confirm enhanced apoptosis-mediated cell death in both animal models. Coupled deletion of Gpx4 and Keap1 completely reverses CCl4-mediated liver damage. Both serum and histological analyses showed no difference between control and dual-knockdown animals.

Conclusion Our results demonstrate that manipulating the ROS response by deleting Keap1 reverses the acute liver damage in hepatocytes with Gpx4 deletion. These data suggest that the deleterious effects of the inactivation of the GPX4 antioxidant system can be rescued by the induction of another cellular mechanism against ROS.

P1.23 Acceleration of an advanced NASH model by acute and toxic effects of Phenobarbital

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Non-alcoholic steatohepatitis (NASH) is an increasing cause for liver cirrhosis globally. NASH-cirrhosis is responsible for liver related complications and no specific treatment is currently available. Animal models for the development of new therapeutic approaches are an unmet need.

The aim of this study was to develop ananimal model of advanced NASH-cirrhosis in rats mimicking the human situation in a shorter period of time.

Liver cirrhosis was induced by repetitive carbon tetrachloride (CCl4) injections in combination with high fat and high-cholesterol western diet (WD). To boost liver injury animals received Phenobarbital in the drinking water using a short-term (ST) high (3d of 0.3g/L) or a long-term (LT) low dose (6 wks of 0.06g/L) treatment.

Rats developed advanced NASH-cirrhosis characterized by blood biochemistry, hepatic steatosis, inflammation and fibrosis. However, LT rats showed ascites as a definite sign of portal hypertension after around 6 weeks, whereas ST rats developed ascites after a median of 8 weeks. All rats showed increased portal pressure and concomitantly a decreased systemic arterial pressure compared to CCl4 alone. Whereas all rat models develop NASH cirrhosis, only rats with LT treatment developed hepatocyte ballooning, which is a sign of parenchymal cell damage/death and also present in human NASH cirrhosis.

The LT administration of low dose Phenobarbital in combination with CCl4 intoxication and WD represents a novel rat model with accelerated development of advanced liver fibrosis mimicking all key characterisitics of decompensated NASH-cirrhosis in humans.

P1.24 ERRγ-induced hepatokine, FGF23, regulates chronic alcohol-induced liver injury in mice

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DOI 10.1055/s-0042-1759929

Circulatory levels of fibroblast growth factor 23 (FGF23), a bone produced hormone responsible for phosphate homeostasis and vitamin D metabolism, is progressively increased in renal diseases and positively correlates with poor prognosis and mortality. Recent reports showed that the liver also produces FGF23 under pathological conditions, however the outcome of hepatic FGF23 remains nebulous. Here, we examined the role of hepatic FGF23 in chronic alcohol related liver disease in mechanistic detail. In mice, chronic alcohol feeding leads to liver damage and simultaneously induces estrogen related receptor γ (ERRγ) and FGF23 gene expression in liver, but not in other major organs. FGF23 gene expression is i transcriptionally regulated by ERRγ in response to alcohol arbitrated activation of cannabinoid receptor type 1 (CB1R). Alcohol induced upregulation of hepatic FGF23 gene expression and plasma FGF23 levels were abolished in hepatocyte specific ERRγ knock-out (ERRγ-LKO) mice, and an inverse agonist mediated inhibition of ERRγ transactivation significantly improved alcohol feeding mediated liver damage and hepatic steatosis in WT mice. Moreover, hepatic Cyp2e1 gene expression is upregulated by FGF23 in response to alcohol. Interestingly, hepatocyte specific knock-out of FGF23 (FGF23-LKO) decreased alcohol induced hepatic Cyp2e1 gene expression and improved chronic alcohol feeding-induced liver injury through inhibition of apoptosis and ROS generation. On the basis of these results, we conclude that the CBIR-ERRγ-FGF23 axis facilitates chronic alcohol mediated liver injury and that the hepatokine FGF23 represents a novel target for its treatment.

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Poster Visit Session ll Clinical Hepatology, Surgery, LTX
27/01/2023, 14.35 pm – 15.10 pm

P2.01 Epidemiology of Liver transplantation and post-LT complications in Germany: nationwide population-based study (2005 to 2018)

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Background Liver transplantation (LT) is the only cure for cirrhosis and acute liver failure. However, LT patients show a considerable in-hospital mortality rate, and different comorbidities may follow and further increase mortality and morbidity after LT. We aim to investigate the trends and changes of outcomes in patients hospitalized for LT or thereafter in Germany in this study.

Methods This German nationwide study investigated the number of admissions of patients hospitalized for LT, and related comorbidity and complications between the year of 2005 and 2018 based on the DRG system with ICD-10 and OPS codes.

Results 12,836 hospital admissions during which patients underwent LT over 14 years were recorded. The in-hospital mortality rate decreased over time. Especially in patients with acute liver failure, the rate declined from 34.9 % in 2005 to 18.9 % in 2018. Despite the decreasing number of LT, admissions of post-LT patients for complications increased from 5984 in 2005 to 11,119 in 2018. Especially complications of immunosuppression had the highest number and nearly tripled in 2018 (7,290) compared to 2005 (2,748), followed by circulatory diseases and biliary complications. Post-LT patients with acute kidney injury (AKI) and biliodigestive anastomosis showed the highest in-hospital-mortality rate of 20.2 % and 18.4 %.

Conclusion In conclusion, our analysis of the nationwide database of all hospital admissions of patients had LT or post-LT in the last 14 years, revealed decreasing mortality rate, but with increasing number of different complications. Biliodigestive anastomosis and AKI are associated with highest in-hospital mortality.

P2.02 Serum proteomics to characterize adult patients with Acute Liver Failure

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Background and aims Acute liver failure (ALF) indicates sudden hepatocellular dysfunction with coagulopathy in patients without known liver disease. Liver transplantation represents the only effective treatment, but the decision for/against transplantation remains challenging. We used mass spectrometry to characterize changes occurring in ALF and to identify prognostic factors.

Methods Serum proteomic patterns were compared in 200 and 119 (discovery/validation cohort) adult patients with ALF, ~ 50 % of them were acetaminophen (APAP)-related as well as in 30 liver-healthy controls. The former were randomly selected from the admission samples (< 48h) of the US ALF study group database. Non-survivors were defined as patients who passed away or required liver transplantation within 21 days. Ingenuity pathway analysis (Qiagen) was used to obtain mechanistic insights.

Results In the discovery cohort, 187 proteins were detected in ≥ 70 % of subjects and displayed aetiology-specific patterns. The majority of them differed between ALF cases and controls. The alterations pointed towards IL-6 signalling, acute phase response and prothrombin activation. 158 proteins differed between APAP and non-APAP cases, three of them reliably and reproducibly discriminated between the groups (AUROCs > 0.9 in both cohorts). In the discovery cohort, 46 proteins varied significantly between survivors and non-survivors. Higher alpha1-antitrypsin and leucine-rich alpha-2 glycoprotein 1 levels associated, aetiology-independently, with better prognosis. In both cohorts, they constituted the best discriminators (AUROCs > 0.7) and were comparable to MELD score.

Conclusion Shotgun proteomics discriminates between different ALF aetiologies and offers prognostic insights.

P2.03 Eine präoperative TIPS-Anlage ist bei Patienten mit Leberzirrhose insbesondere vor Hochrisikoeingriffen mit einer niedrigeren in-house-Mortalität assoziiert

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Fragestellung Patienten mit Leberzirrhose weisen eine erhöhte postoperative Mortalität auf. Ob die Senkung der portalen Hypertension durch eine TIPS-Anlage einen Effekt auf das postoperative Outcome hat, ist unklar.

Methode Retrospektive Datenbankanalyse des TIPS-Kollektivs des Universitätsklinikums Hamburg-Eppendorf zwischen den Jahren 2010 und 2020. Alle Patienten mit einer Leberzirrhose, die innerhalb von 3 Monaten nach einer TIPS-Anlage operiert worden sind, wurden eingeschlossen. Patienten mit Leberzirrhose, die ohne vorherige TIPS-Anlage operativ versorgt wurden, bildeten die Kontrollkohorte. Mittels eines multivariablen Cox propotional hazards model wurden die Variablen Alter, Geschlecht, ASA-Score, TIPS, Art der Operation, Kreatinin, Aszites zum Zeitpunkt der Operation, MELD- und CLIF-Score auf den primären Endpunkt der in-house Mortalität untersucht.

Ergebnisse Insgesamt wurden n = 64 Leberzirrhosepatienten mit und n = 131 ohne TIPS identifiziert, die im genannten Zeitraum operiert wurden. Das Child-Pugh-Stadium in beiden Gruppen war ähnlich, Patienten mit TIPS wiesen aber einen signifikant höheren präoperativen MELD-Score auf (14 vs. 11 Punkte). In beiden Gruppen waren Niedrig- und Hochrisikoprozeduren mit jeweils 47 bzw. 53 % gleich verteilt. Patienten, die mit TIPS operiert wurden, wiesen eine niedrigere in-house Mortalität auf (19 vs. 41 %). In der multivariablen Analyse waren der MELD-Score und Hochrisikoeingriffe mit einem erhöhten, eine TIPS-Anlage mit einem niedrigeren Sterblichkeitsrisiko assoziiert (hazard ratio 0.41, 95-%-Konfidenzintervall 0.18-0.92, p = 0.031). In der Subgruppenanalyse zeigte sich dies insbesondere durch eine geringere Mortalität bei Hochrisikooperationen mit Darmresektionen (TIPS-Kohorte: Mortalität 4/11, 36 % vs. 24/36, 67 % in der Kontrollkohorte).

Schlussfolgerung Eine präoperative TIPS-Anlage ist bei Leberzirrhosepatienten mit einer niedrigeren postoperativen Sterblichkeit assoziiert. Diese Strategie sollte in einer prospektiven randomisierten Studie untersucht werden.

P2.04 Leberfibrose ist assoziiert mit einer schlechteren Gesamtüberlebenszeit und einer höheren Rezidivrate bei Patienten mit Cholangiokarzinom

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Hintergrund Das Cholangiokarzinom ist weltweit der zweithäufigste primäre Lebertumor mit deutlich steigender Inzidenz in den letzten Jahrzehnten. Ziel dieser Studie ist es, den Einfluss der Leberfibrose auf das Gesamtüberleben und die Rezidivrate bei Patienten mit einem Cholangiokarzinom nach Leberresektion zu untersuchen.

Material & Methoden Alle Patienten mit intrahepatischem oder perihilären Cholangiokarzinom, die zwischen Januar 2007 und Dezember 2020 in kurativer Absicht an unserem Zentrum an der Leber operiert wurden, wurden in diese retrospektive Studie eingeschlossen. Es wurden die klinischen und histopathologischen Merkmale analysiert. Der primäre Endpunkt war das ursachenspezifische Überleben. Sekundäre Endpunkte waren das rezidivfreie Überleben und die Identifizierung prognostischer Faktoren.

Ergebnisse Insgesamt erfüllten 80 Patienten die Einschlusskriterien und wurden in die Analyse einbezogen. Es konnte gezeigt werden, dass das Gesamtüberleben (OS) bei Patienten mit Leberfibrose signifikant verkürzt ist ($p < 0{,}001$). Das mediane OS ist bei Patienten ohne Fibrose dreimal so lang wie in der Fibrosegruppe. Darüber hinaus wurde bei Patienten mit Fibrose ein signifikant kürzeres rezidivfreies Überleben (DFS) beobachtet ($p < 0{,}002$). Weitere Analysen zeigten, dass Fibrose der stärkste unabhängige Faktor mit einem negativen Einfluss auf das OS und das DFS ist. Zudem konnte eine negative Korrelation von Fibrosegrad und Gesamtüberleben bzw. rezidivfreies Überleben identifiziert werden ($p < 0.001$).

Schlussfolgerung Das Vorliegen einer Leberfibrose verkürzt sowohl signifikant das OS als auch das DFS bei Patienten, die wegen eines Cholangiokarzinoms an der Leber operiert wurden. Daher ist es wichtig, nicht nur Patienten mit fortgeschrittener Fibrose perioperativ zu überwachen, sondern auch Patienten mit einem niedrigerem Fibrosestadium.

P2.05 IgG4-Related Diseases: A novel mimicker

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DOI 10.1055/s-0042-1759934

Introduction IgG4-associated cholangiopathy (IAC) is a fibro-inflammatory, steroid-responsive condition and needs to be discriminated from primary sclerosing cholangitis (PSC). The association of IAC and IgG4-related colitis is extremely rare.

Case A 50-year-old man was referred to us due to elevated liver enzymes with predominant cholestatic pattern and complaints of fatigue, weight loss, dark urine. Laboratory tests revealed elevated ALT (88 U/L), AST (69 U/L), GGT (673 U/L), ALP (185 U/L) and normal bilirubin levels. MRCP demonstrated stenosis due to wall thickening in the lower 1/3(rd) of the common bile duct (CBD) and in a segment immediately inferior to it. In order to achieve a definitive diagnosis, EUS and ERCP which revealed CBD wall thickening (2,5-3 mm) were carried out. The biopsies were compatible with IgG4-associated cholangitis with > 50 IgG4-positive plasma cells per HPF. He was put on Budesonide treatment. 6-month follow-up was normal until bloody diarrhea appeared. Colonoscopy disclosed fragile colonic mucosa from the descending colon to cecum. However, rectum and sigmoid colon were partially preserved. Histopathological evaluation showed IgG4-related colitis and demonstrated 47 IgG4-positive plasma cells per HPF in inflamed areas.

Conclusion The cause of biliary strictures in young adults is most likely PSC, while in elderly it is cholangiocarcinoma. Our case documents the fact that IAC should also be considered in middle-aged to elderly individuals. Due to capability of affecting multiple organs; individually or synchronous manifestations may appear. To our knowledge, it is the first case that speculate the relationship between IAC and IgG4-related colitis TASL.

P2.06 Diclofenac and Ibuprofen in Patients with Chronic Liver Disease and Liver Cirrhosis

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Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most prescribed medications around the world. Also, they are one of the top sources of drug-induced liver injuries. Several reports studied the effects of Aspirin, but reports on other NSAIDs are widely lacking. Therefore, the aim of our retrospective study was to analyze the effects of diclofenac and ibuprofen administration in patients with chronic liver disease from two large cohorts in South Korea and the United States. We analyzed data from men and women diagnosed with different chronic liver disease (CLD) subgroups. Liver Cirrhosis (LC) 32366 patients, Nonalcoholic Liver Disease (NLD) 27444 patients, Chronic Viral hepatitis B and C (CNV) 21046 patients, and Autoimmune Liver Disease 2728 patients). Investigating the use of diclofenac and ibuprofen did not lead to any disadvantage in survival for Asian patients (LC, $p < 0.001$). Results from the US cohort may even suggest a benefit of these drugs in most patients with chronic liver disease (LC, $p < 0.001$; NLD $p < 0.001$; CVH, $p < 0.001$). However, for Asian patients with chronic viral hepatitis and documented use of either one of these drugs we observed a decreased overall survival (OS) over 15 years ($p < 0.05$). The reasons for the special susceptibility of this population need to be further investigated. With the exception of Asian patients with chronic viral hepatitis B and C, our large cohort data suggests that the use of diclofenac and ibuprofen in patients with chronic liver disease may generally be safe.

P2.07 Posttransplant lymphoma in liver transplantation: A case report

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Objective Transplantation of solid organs has been successful in large part due to the development of immunsupresif regimens that have controlled the recipient's immune system from rejecting the allograft.However,the penalty of the nonspecific nature of immunsupression is the susceptibility of the recipient to the development of opportunistic infections, as well as the increased risk of developing malignencies.

Case A 55 year old male patient underwent liver transplantation in 2015 due to cryptogenic liver cirrhosis.As immunsupressive treatment ;tacrolimus and mycophelolate mofetil treatment was started.The patient presented to the emergency department in February 2021 with abdominal pain and no stool output.The patient was hospitalized due to the detection of air fluid levels. Abdominal CT detected a central necrotic mass of 130 * 90 mm filling the cecum/ terminal ileum.In colonoscopy, ulcerovegetan malignant mass is seen in cecum extended to terminal ileum and biosises were taken.The biopsy result was evaluated as malignant lymphoma.The patient underwent right hemicolectomy due to ileus.Biopsy result was reported as high grade B lymphoma.As a result 6 cycles of R-CHOP treatment was given.

Conclusion Posttransplant lymphoma is a heterogenous group of disease and has a broad spectrum of diseases ranging from early lessions.The most common type of monomorphic posttrasplant lymphoma is Diffuse B large cell lymphoma

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in our case.Posttransplant lymphoma after transplantation has been reported in a rare cases in the literature.However, in recent years, promising results have been obtained with the use of immunomodulatory approaches such as anti CD-20 monoclonal antibody(rituximab), IL-6 and adoptive immunotherapy

P2.08 Ferula Communis-Associated Liver Toxicity: A Case Report

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Introduction Herbal and dietary supplements which are responsible of 15 to 20 percent of the cases of drug-induced liver injury (DILI) are an essential and challenging cause of liver injury. Currently, more than 1000 drugs, toxic agents, herbal products and nutritional supplements are identified with a hepatotoxic effect. Ferula communis (FC), believed to have health benefits, is grown in Eastern Anatolia, Southeastern Anatolia and the Mediterranean Region of our country.

Case A 47-year-old patient presented to the emergency department with diarrhea, nausea and vomiting. This occurred the day after the consumption of a bowl of salad with a herb named FC. His past health was unremarkable and did not use any medications. Physical examination was normal except abdominal tenderness. Laboratory tests showed elevated ALT [1884 IU/L (Normal, 12–63)]; AST [1121 IU/L (Normal,10–37)]; LDH [1103 IU/L (Normal, 85-227)] and impared hepatic synthetic function [INR 1,5 (Normal, 0,85-1,25)]. Laboratory studies revealed negative HAV, HBV, HCV, CMV and EBV serologies. An abdominal ultrasound revealed mild increased liver echogenicity, periportal oedema suggestive of acute hepatitis. The patient was hospitalized with a diagnosis of likely FC induced toxic hepatitis. Treatment with intravenous N-acetylcysteine and hydration was initiated. A gradual decrease in altered liver function tests was observed.

Conclusion Patients diagnosed with acute hepatitis should be questioned about herbal additives. Sharing this kind of knowledge is essential to increase awareness of herbal toxicities, make early diagnosis and prevent progression to severe liver damage. TASL.

P2.09 Drug-induced liver injury presenting with hypereosinophilia and increased FDG activity in PET scan

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Introduction Drug induced liver injury accounts for 10 % of all cases of acute hepatitis. Over 1.000 medication and herbal products have been implicated. The clinical presentation includes a variety of clinical and pathological phenotypes.

Case A 56-year-old patient was referred to our clinic with a 3-month history of cough, a 1-week history of jaundice and fatigue. Additionally, a Thorax Ct scan which revealed 1.5 cm nodular lesion and 1 cm mediastinal lymphadenopathies and a PET Scan with FDG uptake of the lung nodule, spleen and bone marrow and rectum were performed. He denied abdominal pain, fever, nausea, diarrhea and vomiting. In last 3 months he had a travel history to Phuket, New York, Israel and Germany. He started taking several herbals 2 months ago. Laboratory tests revealed eosinophilia (1080 UL), leucocytosis (28,900/mm3), elevated levels of ALT (1994 U/L), AST (1331 U/L), ALP (221 U/L), GGT (182 U/L), bilirubin (Total/direct bilirubin: 10.1/7.4 mg/dL,), INR (2.38), ESR (68 mm/hr). Peripheral blood smear confirmed hypereosinophilia. Serology for viral, parasitic infections and autoimmune hepatitis were negative. Bone marrow and liver biopsy disclosed hypercellular bone marrow with increased eosinophils and erythroid cells and acute hepatitis with centrilobular and bridging necrosis, plasmocyte and eosinophil rich inflammation in the portal zone, respectively.

Conclusion Since all medications usually underreport by the patients, the diagnosis of DILI requires a high degree of awareness and suspicion after excluding other causes of abnormal liver tests TASL.

P2.10 Natural Dısease Outcome In Patıent Wıth Nonalcoholıc Fatty Lıver Dısease: A Study Wıth Paıred Lıver Bıopsıes

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Backround The aim of the study was to assess relationship between histological features and disease outcome in patients with non-alcoholic fatty liver disease(NAFLD) using paired liver biopsies.

Methods This is a single-center study.Between January 2001 and December 2021, we reviewed 116 patients(male/female:61/55) with NAFLD,who had paired sequential liver biopsies. Biopsy samples were assessed using NASH CRN Scoring system. Significant fibrosis was defined as > = stage 2 fibrosis. The median follow-up period was 123 months.

Results Patients median age was 51 years, and 22 % had diabetes mellitus (DM), 66 % had hyperlipidemia(HL) and 33 % had hypertension(HT). Three patients had a history of cardiovascular disease(CVD). Median serum AST, ALT and GGT levels were 35.5U/L (16-146), 57U/L (17-352) and 47.5U/L (17-352). Eighty-seven patients (75 %) had hepatic fibrosis at baseline biopsy: 20(17 %) had significant fibrosis (> = F2), 9(8 %) had advanced fibrosis (F3 and F4), and 2 patients were compensated cirrhosis. The median interval between two biopsies was 33 months. From baseline to the end of the follow-up period, fibrosis regressed in 22 patients, no changed in 66 patients and progressed in 28 patients (▶**Table 1**). The proportion of significant fibrosis was increased from 17 % to 25 %. Nine patients developed new-onset cirrhosis: 5 of them had significant fibrosis at their baseline biopsies. Three patients had a new-onset decompensation event. New-onset DM was developed in 28(24 %) patients, HL in 21(18 %) patients, and HT in 19(16 %) patients. A total of 13(9 %) patients had a history of a CVD.Extrahepatic malignancy developed in 12 patients, whereas hepatocellular carcinoma developed in two patients. Six patients died.

In Conclusion NAFLD is a potentially progressive disease and is associated with hepatic and extrahepatic events.

P2.11 Trientine tetrahydrochloride versus DPA for the management of patients with Wilson Disease: Results from the CHELATE Trial

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Background and Aims Treatment of Wilson Disease (WD) with chelating agents [d-Penicillamine (DPA) or trientine] prevents the pathologic accumulation of copper. Up to now, there are no prospective controlled studies comparing DPA with trientine. The aim of the study is to determine if maintenance therapy of adults with WD using trientine tetrahydrochloride formulation (TETA4) is safe and effective compared to DPA.

Method Multicentre, non-inferiority-controlled trial comparing TETA4 with DPA in clinically stable adult WD patients on DPA.

Patients were randomised to either continue DPA therapy or switched to TETA4. Treatment efficacy was evaluated from repeated measures using serum NCC, UCE and blinded clinical. Subjects were monitored by laboratory and clinical evaluation, including neurological assessment. After the primary endpoint at 24wk, subjects entered an extension phase for further 24wk.

Results Mean difference in serum NCC after 24wk and 48wk was −9.1 (−24.2, 6.1) µg/L and – 15.5 (−34.5, 3.6) µg/L respectively. At 24 wk post randomisation UCE was lower with TETA4. UCE at 48 weeks was not significantly different. At baseline and at 48wk, UWDRS score was 14.2 (23.2) vs. 8.3 (11.4) and 14.2 (18.3) vs. 8.8 (7.8) for DPA and TETA4 respectfully. Data on liver tests were all either not clinically or statistically different from baseline. There were 3 post-randomisation SAEs with DPA; none for TETA4. All AEs resolved and were mild to moderate.

Conclusions TETA4 is effective and well tolerated in the maintenance therapy in adults with WD, and non-inferior to DPA.

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P2.12 Use and outcome of transjugular intrahepatic portosystemic shunt in Germany: nationwide population-based study (2007 to 2018)

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Objective Portal hypertension(PHT) is the driver of many complications of liver cirrhosis. An increasing number of cirrhosis and also complications of PHT have increased overtime. Transjugular intrahepatic portosystemic shunt(TIPS) placement is the most effective treatment of PHT. The aim of this study was to analyze the use and impact of TIPS placement in patients with different indications and the relevant complications over the last decade in Germany in a nationwide study.

Design All hospital admissions in Germany from 2007 to 2018 were analyzed using the DRG system. All diagnosis and procedures were coded according to the ICD-10-CM and OPS code.

Results Between 2007 and 2018, there were 14,598 admissions of patients for TIPS insertions, of which the vast majority(88.7 %) of patients had cirrhosis. In patients with bleeding, TIPS did not significantly decrease their in-hospital mortality(22 %). Patients with ascites (in-hospital-mortality: 8.7 % with TIPS vs.15.1 %) and hepatorenal syndrome(in-hospital-mortality: 17.1 % with TIPS vs.43.3 %) benefited the most from TIPS insertions. About 20 % of patients with TIPS insertion developed a hepatic encephalopathy(HE). However, TIPS improved the survival of patients with HE grades 1 and 2 by 10 % and 33 %, respectively. Moreover, significant survival benefit through TIPS insertions could be found in patients with circulatory diseases (in-hospital-mortality: 8.6 % with TIPS vs.10.4 %) or infections (in-hospital-mortality: 18.2 % with TIPS vs.20.5 %) as comorbidities of cirrhosis.

Conclusion Our nationwide population-based study demonstrates that TIPS-insertion is widely used and leads to control of complications of PHT. Most patients receiving TIPS have a lower hospital mortality and less circulatory and infections complications.

P2.13 Antibiotics use in patients with chronic liver disease

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Antibiotics are commonly prescribed in patients with chronic liver disease (CLD). However, the use of antibiotics in patients also raises concerns for many physicians, particularly drug choice, eventual dose adjustments, or toxicity in patients with CLD. Therefore, we performed a retrospective review of 70,632 patients with CLD from two large cohorts in the United States and South Korea to analyze the effects of antibiotic use on these patients. Data on antibiotic use in these patients from 2000 to 2020 were collected. These data were compared with patients' overall survival to explore the impact of antibiotic use on the clinical outcome of patients with CLD. A total of 16 antibiotics were evaluated. All 13 drugs investigated in the Asian cohort were associated with a worse prognosis (p = 0.000) except for Neomycin (p = 0.123). Furthermore, in the US cohort, highly potent antibiotics mainly used for the treatment of multi-drug resistance organisms, healthcare-acquired or nosocomial infection, e.g. Amphotericin B, Vancomycin, and Imipenem were also associated with a worse prognosis (all p = 0.000). In contrast, antibiotics primarily used for mild infections and outpatient treatment, e.g. Ofloxacin, Ciprofloxacin, and Azithromycin (p = 0.000), were even associated with a better outcome for the US cohort. Overall, our large real-world data suggest that the need for antibiotics aimed at multi-drug resistance organisms, and healthcare-acquired or nosocomial infection is an indicator for worse outcomes in patients with CLD. Different outcomes associated with the use of antibiotics primarily used for mild and outpatient infections need to be further evaluated.

P2.14 Stage of fibrosis is not a predictive determinant of weight loss in patients undergoing bariatric surgery

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Background Obesity and Non-alcoholic fatty liver disease (NAFLD) are an increasing health care burden, worldwide. Weight loss is currently the best option to alleviate NAFLD and is efficiently achieved by bariatric surgery. Presence of NAFLD seems to be predictive for postoperative weight loss. To date, only few predictive factors for post-bariatric weight loss (age, diabetes, psychiatric disorders) are established. Since liver fibrosis is the pathogenic driver for the progression of liver disease, we investigated its role in predicting postoperative weight loss. This study focuses on the correlation the correlation between fibrosis stage and weight loss.

Methods We used a prospective, single-center cohort study including 164 patients who underwent bariatric surgery with simultaneous liver biopsies. Liver fibrosis was determined histologically according to Kleiner score and non-invasively by APRI and FIB-4 score. Percentage of total body weight loss was calculated at one-year follow up visit.

Results 32 patients were found without fibrosis, whereas 91 patients showed mild fibrosis (F1), 37 significant fibrosis (F2), and only four patients presented advanced fibrosis (F3) at the time of bariatric surgery. Weight loss was similar across different degrees of fibrosis stage. Accordingly, linear regression analysis did not identify predictors of weight loss among fibrosis scores. In multivariable analysis, age and presence of diabetes showed the strongest predictive value.

Conclusions Baseline presence of fibrosis was not associated with postoperative weight loss, while age and diabetes were independent predictors of weight loss. Bariatric surgery should be applied independently of the fibrosis stage.

P2.15 Boosting compromised SARS-CoV-2-specific T cell immunity in liver transplant recipients by mRNA vaccination

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Background and aims Liver transplant recipients (LTR) are threatened by a lower immunogenicity of SARS-CoV-2 mRNA vaccines. However, the interplay between the different branches of the adaptive immune system especially after a third (and fourth) vaccine dose is still poorly understood.

Methods Our study longitudinally compares the humoral as well as the cellular response between age-matched LTR (n = 24) and healthy controls (HC, n = 19) after three to four vaccine doses. Therefore, we assessed antibody titers, analyzed the spike-specific T cell epitope repertoire, performed an in-depth characterization of spike-specific CD8 + T cells on a single-epitope level and examined the distribution of different virus-specific CD4 + T cell subpopulations.

Results Compared to HC, the development of high antibody titers depended on a third vaccine dose in most LTR. In contrast, spike-specific CD8 + T cells reached a stable level already after the second vaccine dose, albeit with a lower frequency and a narrower epitope repertoire compared to HC. Concerning the CD4 + T cells, the total number of detectable responses as well as the repertoire of targeted epitopes within the spike protein did not significantly differ in both cohorts. However, we observed a link between the overall attenuated vaccine response and a reduced frequency of spike-reactive follicular T helper cells (TFH) in LTR.

Conclusion Three doses of a COVID-19 mRNA vaccine induce an overall robust humoral and cellular memory response in most LTR. Evaluations of additional booster doses may thus consider the individual vaccine responsiveness as well as the evolution of novel variants of concern.

P2.16 Common apolipoprotein E (APOE) variant in is associated with increased liver injury in patients with autoimmune hepatitis

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Background The course of autoimmune hepatitis (AIH) varies between patients. Recent analyses linked the apolipoprotein E (APOE) variant rs429358 with steatosis and liver injury in NAFLD (Jamialahmadi et al., Gastroenteorology 2021) whereas we demonstrated the MARC1 variant might decrease liver injury in AIH. Here, we analyse this APOE variant in patients with AIH.

Methods The study cohort consisted of 312 non-transplanted (70 % women, age 18–83 years, 130 with cirrhosis, 60 therapy-naïve) adults with AIH. The APOE rs429358 polymorphisms was genotyped using TaqMan assays. Liver fibrosis was assessed by liver biopsy, liver stiffness and serum indices (APRI, FIB-4), liver steatosis was evaluated by controlled attenuation parameter (CAP).

Results At least one copy of the APOE minor allele was present in 26 % of patients with AIH. The APOE polymorphism was associated with more pronounced liver fibrosis according to APRI score (P = 0.035) and higher AST (P = 0.049) as well as ALT (P = 0.055) activities in therapy-naïve patients. A trend between the genetic variant in APOE and higher ALT was also present in the entire cohort (P = 0.054). On the other hand, this variant did not affect the results of liver biopsies, transient elastography or CAP (P > 0.05). The protective effect of the MARC1 genotype on serum ALT (P = 0.045) and AST (P = 0.024) were stronger in patients with APOE wild-type genotype.

Conclusions Patients with AIH who carry the APOE polymorphism might manifest a more pronounced liver injury. Genotyping of the APOE and MARC1 genetic variants might help to stratify patients with AIH concerning their risk of progressive liver injury.

P2.17 Invasive fungal disease of the liver transplant – an underestimated burden, contributing to chronic transplant failure

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Introduction Invasive mycosis is associated with high morbidity and mortality in solid organ transplant patients and represents an important clinical and diagnostic challenge. In order to elucidate the incidence and the significance of invasive mycoses in chronic liver transplant failure (CLTF), a retrospective clinicopathological study was performed at Heidelberg-University Hospital in cooperation with the Tissue bank and the Liver Transplant Cohort of the German Center for Infection Research.

Materials and methods The study collective included all cases of CLTF (≥ 90-day graft survival) of Heidelberg University Hospital from 1991–2021 (n = 154). Archival tissue samples from the explanted liver transplants were comprehensively reassessed. Additional stains (Grocott methenamine silver, Periodic Acid-Schiff (PAS)) were performed, whenever applicable, and all correlating morphological parameters (semiquantitative bile duct involvement and damage, inflammation, concrement formation) were evaluated.

Results Fungal infection was present in 42/154 (27,3 %) cases (28 newly detected). It affected the main and interlobular ducts with a strong association with bile duct destruction, biliary concrement and features of acute (abscess formation) and chronic inflammation (fibrosis). The standardized use of special stains resulted in the detection of additional cases, mostly with more limited disease and lower number of fungal organisms.

Conclusion Considering the significant portion (more than 1/4) of fungal infection in our cohort, the incidence and relevance of fungal infection in CLTF is underrated and deserves interdisciplinary attention; our study describes the morphologic patterns and paves the way for further clinical, serological, and molecular analyses in order to improve diagnosis, prevention, and outcome of invasive transplant mycosis.

P2.18 Short chain fatty acids in stool correlate with quality of life and markers of liver injury in patients with NAFLD: preliminary results

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Introduction Patients with non-alcoholic fatty liver (NAFLD) have impaired life quality (LQ). Previous studies alluded to an association between intestinal microbiome and life quality (Lee et al., Cell. 2022). Here we investigate the association between the metabolites secreted by the intestinal bacteria, namely short-chain fatty acids (SCFA), and LQ in patients with fatty liver.

Methods Adult patients with different stages of fatty liver were recruited in our outpatient clinic. Hepatic fibrosis and steatosis were quantified non-invasively using transient elastography (TE) and controlled attenuation parameter (CAP), respectively. Life quality was measured using the PHQ-9, GAD-7, FIS-D, and SF-12 questionnaires. SCFA were measured in stool samples from 15 patients by gas chromatography. Genetic risk factors for NAFLD: PNPLA3 p.I148M, MBOAT7 rs641738, and TM6SF2 p.E167K were genotyped using TaqMan assays.

Results In total 79 patients (29 males, age range 23–78 years) were included: 17 showed moderate to severe depression and 10 had anxiety, 6 had FIS-D values indicating fatigue. In particular women had more often severe depres-

sive symptoms. Stool acetic acid correlated with serum AST (p = 0.007) and with liver stiffness(p = 0.009); isovaleric (p = 0.014), valeric (p = 0.003) and isobutyric (p = 0.016) acids correlated with serum ALT. Stool propanoic acid correlated with depression according to PHQ-9 (p = 0.011). Among genotyped variants, the MBOAT7 polymorphism was associated with acetic (p = 0.017) and and butyric (p = 0.018) acids in stool.

Discussion Our preliminary results indicate potential links between stool SCFA, liver injury and LQ in patients with NAFLD. These associations seem to be modulated by the genetic predisposition.

P2.19 Ascitic calprotectin – An early biomarker in spontaneous bacterial peritonitis

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Background Spontaneous bacterial peritonitis (SBP) is a severe complication of decompensated liver cirrhosis. Diagnosis is assured with detection of > 250 polymorphonuclear (PMN) cells per µl ascitic fluid. Since antibiotic treatment is indicated at the very onset of SBP, innovative potential biomarkers for early diagnosis are of great clinical relevance.

Methods To identify novel potential biomarkers for SBP, ascitic fluid was collected from patients with liver cirrhosis treated at a German University Hospital. Calprotectin protein was determined by ELISA. Total protein was measured by BCA assay.

Results 190 ascitic fluid samples from 128 individual patients were analyzed. SBP was diagnosed in 29 (15 %) samples. Ascitic fluid samples (calprotectin range 27-163.59 µg/ml) with SBP had higher ascitic calprotectin levels (median 8999 ng/ml) and calprotectin-total-protein-ratios (median 5x10-4) in comparison to samples without infection (median 539 ng/ml; ratio: median 3x10-5). Ascitic calprotectin (r = 0.7; p < 0.001; cut off: 4.4 mg/ml sensitivity 83 %, specificity 98 %) and calprotectin-total-protein-ratio (r = 0.7; p < 0.001; cut-off 208x10-6 sensitivity 79 % specificity 93 %) significantly correlated with ascitic PMN cell count. Time course analyses in follow-up paracentesis revealed that development of SBP was associated with an increase of calprotectin-total-protein-ratio, which was detectable before PMN count exceeded 250. Accordingly, calprotectin-total-protein-ratios decreased with recovery from SBP.

Conclusion Ascitic calprotectin-total-protein-ratio correlates with state of the art diagnostic criteria, is associated with time course and recovery from SBP and is a potential predictive marker for early SBP. These results provide options for earlier diagnosis and treatment of SBP by measurement of ascitic calprotectin.

P2.20 Partial splenic embolization used for rescue treatment for variceal bleeding as a safe and efficient procedure – experience at a university medical center

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Background Partial splenic embolization (PSE) is a non-surgical procedure, currently used to treat different clinical diseases and conditions such as portal hypertension and esophageal and gastric variceal hemorrhage due to portal hypertension and/or splenic vein thrombosis. We evaluated the safety and efficacy of PSE in patients with variceal bleeding due to portal hypertension.

Methods Within seven years (2015-2022), twenty-five patients with esophageal varices grade III-IV or gastric fundal varices underwent PSE. All patients had significant portal hypertension due to different underlying diseases. In two cases patients with hypersplenism and cytopenia PSE was preferred despite technical feasibility of TIPS placement.

Results All patients with acute or recurrent esophageal and gastric fundal variceal bleeding were successfully treated with PSE as rescue therapy. In twelve cases with uncontrollable variceal bleeding PSE was performed under emergency conditions. Control gastroscopy revealed significant regression of esophageal and gastric varices, classified as Grade II or lower after PSE. During the follow up period no further bleeding episodes occurred. Platelet count increased starting from day one after PSE. After one week, thrombocyte levels improved significantly. After six months, thrombocytes maintained at significantly higher levels. Fever and abdominal pain were side effects of the procedure. Severe complications were not observed.

Conclusion PSE is a successful salvage therapy for patients with esophageal and/or gastric varices in which endoscopic treatment options or placement of a transjugular intrahepatic portosystemic shunt are not promising or technically not feasible. Even for patients with fulminant variceal bleeding PSE is an effective tool in emergency treatment.

P2.21 Interprofessional collaboration between physicians, nurses, and pharmacists improves medical and economical outcomes at an intensive care unit with focus on hepatology

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Background and Aims Since 2015, the medical intensive care unit (ICU) with a focus on hepatology of the Department of Internal Medicine 1 at the University Hospital Regensburg, Germany, has a particular emphasis on interprofessional collaboration with staff nurses and hospital pharmacists. Furthermore, there is a joint training and teaching of medical, nursing and pharmacy students within the intensive care training ward Regensburg (I'M A-STAR project). The study aims to investigate to what extent the newly introduced structural changes affect clinical and economic outcomes.

Method We examined clinical performance data and consumption figures for antibiotics and other drugs over a 10-year period from 2011 to 2021. An electronic platform was developed specifically to improve documentation. The years 2020 and 2021 were considered separately due to the COVID-19 pandemic and the care of numerous COVID-19 patients in the ICU.

Results It could be shown that the pharmacist's recommendations regarding drug administration were mainly related to indication (43.6 %), dosage (27.6 %), interactions (9.4 %), and side effects (4.1 %). Antibiotic consumption was reduced by 12.2 % from 2015 to 2019. Encouragingly, this included a 23.4 % reduction in carbapenem use. Antibiotic spending was reduced by 24.9 % overall. In another analysis, antibiotic spending per case-mix point was calculated. While spending was EUR 60.22 per case-mix point in 2015, this could be reduced by 42.9 % to EUR 34.37 per case-mix point by 2019.

Conclusion Through close interprofessional collaboration between physicians, staff nurses, and pharmacists, the consumption of antibiotics and other drugs was significantly reduced, thus improving patient care.

P2.22 Decompensation Increases The Risk of Cardiovascular Events In Patients With Cirrhosis and Coronary Artery Disease

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Background and Aims The prevalence of coronary artery disease (CAD) in patients with liver cirrhosis is high. The risk of developing cardiovascular disease (CVD) events in patients with cirrhosis and known CAD has not been evaluated yet. Thus, the aim of this study was to evaluate the risk of CVD events and associated predictors in patients with cirrhosis and CAD.

Methods This is a monocenter retrospective observational study. 130 patients with liver cirrhosis and CAD were included in the study. Patients were followed up for CVD events (defined as symptomatic arrhythmia, acute coronary syndrome, new-onset heart failure or cardiac death) and all-cause mortality for 12 months as primary and secondary outcome, respectively.

Results In total, 14 patients (11 %) developed CVD events within 1 year. There was no significant difference in the rate of secondary prophylaxis. Decompensated cirrhosis (CTP B/C) (HR 1.17, 95 % CI1.17-4.68, p = 0.035) as well as MELD (HR 1.13, 95 % CI 1.05-1.22, p = 0.002), were independently associated with development of CVD events. Age (HR 4.81, 95 % CI 1.27-18.28, p = 0.021), left ventricular ejection fraction (HR 0.97, 95 % CI 0.94-0.99, p = 0.039) and c-reactive protein (HR 1.01, 95 % CI 1.00-1.02, p = 0.025) independently predicted 1-year mortality.

Conclusion The risk of CVD event development in patients with cirrhosis and CAD is low. Decompensated stage (CTP B/C and higher MELD) of liver cirrhosis independently predicts development of CVD events.

P2.23 MCAM is a prognostic serum biomarker in patients with liver cirrhosis and HCC

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Chronic liver diseases are a major public health burden. The rising incidence of patients with liver cirrhosis and hepatocellular carcinoma (HCC) emphasizes the need for non-invasive markers that predict poor prognosis. Melanoma cell adhesion molecule (MCAM) is a cell surface adhesion molecule expressed by diverse cell types. MCAM cleavage by metalloproteinases, known to be enriched in fibrotic and malignant diseases, results in release of a soluble form into the serum. The aim of this study was to evaluate soluble MCAM as a prognostic serum biomarker in patients with chronic liver disease. Interestingly, we found serum levels of MCAM to be highly dysregulated in patients with liver cirrhosis and HCC (n = 302 and n = 54, p < 0.0001, F-test, respectively). Corroborating an association of MCAM release with fibrogenesis and carcinogenesis, serum levels correlated with both liver stiffness by transient elastography in patients with liver cirrhosis (p = 0.02) and alpha-fetoprotein (AFP) level in patients with HCC (p = 0.05). Moreover, MCAM serum levels showed an association with overall survival in both study cohorts. Thus, patients with HCC and MCAM serum levels > 1216 ng/ml showed a more than 14 times higher risk of death within 5 year's follow up compared to patients with lower levels (p = 0.001, log-rank test). Collectively, our study reveals MCAM as a novel prognostic serum biomarker in patients with liver cirrhosis and patients with HCC.

P2.24 Secondary sclerosing cholangitis in patients with COVID-19 ARDS – a specific entity associated with high mortality?

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Introduction SSC-CIP (secondary sclerosing cholangitis in critically ill patients) is characterized by biliary tract destruction after long intensive care treatment. Hypotension and vasopressor therapy are main risk factors. Increased prevalence of SSC-CIP occurred in patients with COVID-19 ARDS that were treated by endoscopic retrograde cholangiography (ERC).

Aims The aim of the study was to analyze clinical, laboratory, microbiological and endoscopic findings of patients with SSC-CIP with COVID-19 ARDS.

Methods Data of 17 patients with SSC-CIP with COVID-19 ARDS between February 2020 and August 2022 were analyzed retrospectively. The focus was on endoscopic findings, laboratory and microbiological values and on clinical parameters and potential risk factors during COVID-19 ARDS.

Results 14 male and 3 female patients were included. The mean age was 60 years (range 40–76). All patients were mechanically ventilated, 11 patients were treated with ECMO. All patients required catecholamine therapy but only low dosed when compared with other septic conditions.

On average 2.6 ERCs were performed. Biliary casts were found in 94 % of the patients and rarefication of the intrahepatic bile ducts in 50 %. Bile duct stenosis was detected in 3 patients. Casts were extracted and stenoses were dilated. 13 patients died, 4 patients are in follow-up with repeated endoscopic intervention and re-evaluation in regard to liver transplantation.

Discussion Mortality rate in patients with SSC-CIP with COVID-19 ARDS is high. Vasopressor therapy and hypotension was not prominent in this cohort. Endoscopic treatment may improve liver function, however these patients must be evaluated for liver transplantation.

P2.25 Splenic volume – useful as prognostic factor in patients with portal hypertension after TIPS implantation?

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Background Splenomegaly is a common finding associated with portal hypertension and splenic volume (SV) has been proposed as a prognostic parameter in several liver diseases. Transjugular intrahepatic portosystemic stent-shunt (TIPS) implantation is an established procedure to reduce portal hypertension. We aimed to investigate the impact of true SV and laboratory scores on overall survival (OS) and hepatic venous pressure gradient (HVPG) after TIPS implantation.

Material and methods Between 2010 and 2020, we retrospectively included 199 patients with TIPS and an in-house, contrast-enhanced CT scan prior to TIPS implantation. SV was assessed using a 3D U-Net trained for splenic segmentation and compared against OS, presence of spontaneous splenorenal shunt, and the MELD and ALBI score.

Results In our cohort, SV did not significantly correlate with HVPG (Pearson's r = 0.12, p = 0.09). Patients with a spontaneous splenorenal shunt showed lower SV than those without (258ml/m² ± 110ml/m² vs 357ml/m² ± 223ml/m², p = 0.04). Interestingly, patients with SV above the median (295ml/m²) showed prolonged survival times (1-year-OS 0.78 vs 0.68, 5-year-OS 0.62 vs 0.40, log-rank p = 0.02). Regarding scoring systems, optimal stratification yielded prognostic cut-offs for MELD (cut-off 19, 1-year-OS 0.68 vs 0.79, 5-year-OS 0.47 vs 0.57, log-rank p = 0.04), but not for ALBI (best log-rank p = 0.2).

Conclusion Even though correlation between SV and HVPG was weak, stratification according to SV yielded significantly different survival between groups. While portal hemodynamics and the relationship to splenomegaly has again proven complex, volumetric assessment of SV before and likely SV changes after TIPS implantation might help to early identify patients responding favorably to the procedure.

P2.26 Immunonutritive scoring for survival stratification in patients undergoing transjugular intrahepatic portosystemic shunt: Results from a dual-center study

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Background Immunonutritive scoring has been identified as an additional tool for survival stratification in patients with various cancer entities and chronic diseases. However, immunonutritive scoring has not been evaluated in patients undergoing transjugular intrahepatic portosystemic shunt (TIPS). Furthermore, it remains unclear which of the proposed scoring systems is the most applicable for this patient group.

Methods Between 2010 and 2020, we retrospectively identified 225 patients who underwent TIPS at two tertiary care centers. In these patients, the Prognostic Nutritional Index (PNI) and CRP-albumin-lymphocyte (CALLY) were calculated using albumin, total lymphocyte count, and in the case of CALLY the CRP level, too. Cut-off values were calculated using optimal stratification. Both scores were compared in univariate and multivariate regression analyses, adjusted for established risk factors.

Results While the CALLY was able to significantly stratify patients according to their survival outcome after TIPS (low CALLY: 1-year and 3-year survival rates of 67.8 % and 45.1 %, high CALLY: 1-year and 3-year survival rates of 80.3 % and 73.3 %, log-rank $p < 0.001$), the stratification according to PNI resulted in a trend towards poorer survival with low PNI values but didn't reach significance (low PNI: 1-year and 3-year survival rates of 67.4 % and 54.4 %, high PNI: 1-year and 3-year survival rates of 78.5 % and 64.0 %, log-rank $p = 0.07$).

Conclusion With the CALLY index outperforming the PNI, immunonutritive scoring was able to stratify patient survival after TIPS and might become one additional facet in patient evaluation and follow-up. Furthermore, future studies should evaluate possible combination with existing scoring systems.

P2.27 Influence of NOD2 risk variants on the prevalence of hepatic encephalopathy and association with inflammation, bacterial translocation and immune activation in cirrhosis

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Background and Aims NOD2 risk variants are associated increased bacterial translocation (BT). The aim of the study was to evaluate the association between the presence of NOD2 risk variants, BT, inflammation, and HE (covert and overt).

Patients and Methods This prospective multicenter study included patients with cirrhosis with genetic testing for the NOD2 risk variants and evaluated for covert (C) and overt (O) HE (CFF and clinical). Markers of systemic inflammation (leukocytes, CRP, IL-6, LBP) and immune activation (soluble CD14) as well as bacterial endotoxin (hTRL4 activation) were determined in serum.

Results Hundred seventy-two patients [70 % men; median age 60 (IQR 54-66); MELD 12 (IQR 9-16); 72 % ascites] were included, and 53 (31 %) carried a NOD2 risk variant, 11 % had OHE and 27 % CHE. Presence and severity of HE and surrogates of inflammation, BT and immune activation are shown on table 1 (See table 1). The results did not change after adjustment for MELD. In contrast, HE (both OHE and CHE) was associated with increased systemic inflammation: CRP [w/o HE: 7.2 (2.7-16.7); with HE 12.6 (4.5-29.7) $p < 0.001$] and soluble CD14 [w/o HE 2592 (2275 -3033); with HE 2755 (2410-3456), $p = 0.025$].

Conclusions The presence of NOD2 risk variants in patients with cirrhosis is not associated with the presence of overt and covert HE and has no marked impact on inflammation, bacterial translocation or immune activation. In contrast, the presence of HE was linked to the acute phase response and myeloid

P2.28 Evaluation of coagulation function by ROTEM in pre-/intra-/post-hepatic blood samples during TIPS-implantation in liver disease patients

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Background In patients with liver cirrhosis, transjugular-intrahepatic-portosystemic-shunt (TIPS) is considered a standardized treatment of severe conditions, e.g., refractory ascites or variceal bleeding. TIPS-thrombosis (TT) and/or portal-vein-thrombosis (PVT) are possible complications during/after TIPS placement. Previous studies suggested increased clotting activity in portal circulation (PORC). This pilot study evaluated the PORC and peripheral circulation (PERC) coagulation function using rotational-thromboelastometry (ROTEM) during TIPS.

Methods Blood samples were collected from cirrhotic patients (n = 13) undergoing TIPS from the following compartments: median cubital vein (MCV; PERC) – the confluence of the three hepatic veins to the inferior cava vein (HV/ICV; PORC) – portal vein(PV; PORC) – TIPS (PORC).

Results EXTEM results showed no significant differences for CT [M (70 – 73) ± SD (9 – 13); $p = 0.93$] or CFT [M (137 – 155) ± SD (75 – 112); $p = 0.97$] or MCF [M (51 – 54) ± SD (9 – 10); $p = 0.90$] or ML [M (9 – 10) ± SD (4 – 5); $p = 0.89$) between the compartments. We detected no differences in coagulation function between the PERC and the PORC in patients with cirrhosis using ROTEM.

Conclusion The results of this pilot study contrast with previous reports suggesting increased clotting activity in the PORC vs. PERC in association with liver cirrhosis. ROTEM-based evaluation of coagulation function in PERC appears to reliably reflect coagulation function in PORC with respect to risk estimation for TT and/or PVT among cirrhotic patients undergoing TIPS.

P2.29 Health-Economic Evaluation of a Medical Intermediate Care Unit (MIMC) with Focus on Liver Diseases at a Maximum Care University Hospital.

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Intermediate-care-units (ImCUs) meet the treatment needs of patients with complex diseases and those requiring advanced nursing care; therefore, ImCUs and can make occupancy management of intensive care beds more efficient. Despite exclusion of nursing staff costs from the Diagnosis-Related-Groups (DRG) reimbursement system, prolonged periods of below-average monthly revenues due to misallocation/blocking of ImCU beds and/or loss of complex DRGs can lead to a fixed-costs refinancing problem. Thus, the aim of the present

work was to evaluate the profitability of a liver-diseases-focused ImCU, as part of an interdisciplinary medical IMC unit (MIMC) at the University Hospital Essen, for the period 01.01.2014–31.12.2016. To this end, 1015 cases of the MIMC ward (Department of Gastroenterology and Hepatology, Med.GH./MIMC) were examined with regard to age, and sex (median patient age 57 years; ♂ 61 %, ♀ 39 %), length of stay (LoS), admission/main diagnosis, procedures provided or secondary diagnoses and revenues. Overall, 85.0 % of DRG reimbursements came from cases within the top 20 base DRGs; this finding highlights the hepatological focus of Med.GH./MIMC. The case-mix (CM) monthly average was 65; the CM-index (CMI) monthly average was 10.891. Average LoS on Med.GH./MIMC was 12.3 days, which is significantly longer than the average LoS in German hospitals (7.2 days). Economic assessment of Med.GH./MIMC reveals that the inpatient revenues increased from € 2.90 to 3.72 million (2014 – 2016). Thus, there was a positive development of primary revenues from € 2.98 (2014) to 3.56 (2015) to 3.81 million (2016). This can be considered as an exceptionally good health economic development/outcome.

P2.30 The Role of a Bile Sample-based multiplex real-time PCR Assay (SeptiFast) in Antibiotic Management of Patients with Cholestatic Liver Diseases.

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Real time-Polymerase Chain Reaction (RT-PCR) techniques provide rapid detection of pathogens. This pilot-study aimed to evaluate clinical utility and impact of multiplex RT-PCR (mRT-PCR, SeptiFast) versus (vs) Conventional Microbial Culture (CMC) in bile samples of patients with chronic Cholestatic Liver Diseases (cCLD) undergoing Endoscopic-Retrograde-Cholangio-Pancreatography (ERCP) under periinterventional Antimicrobial Prophylaxis (pAP).

To this end, we prospectively collected bile samples from patients (n = 26) with cCLD during ERCP. Microbiological analysis of the bile was conducted by CMC and mRT-PCR. Krippendorff's-alpha for interrater reliability and Jaccard-Index for similarity describe the concordance of both methods.

mRT-PCRbile and CMCbile showed concordant detection only for Candida albicans (k-alpha: 0.8406, Jaccard-Index: 0.8181). Among patients with Clinical Signs of Infection (CSI), mRT-PCR/bile detected pathogens in 8/8 cases (100 %), CMC/bile in 7/8 cases (87.5 %), and CMC/blood in 5/8 cases (62.5 %). mRT-PCR/bile, CMC/bile and CMC/blood delivered identical results in 37.5 % of cases with CSI (n = 3/8). i.e., 2x Klebsiella spp. and 1x Enterococcus faecium. Compared to CMC, mRT-PCR demonstrated higher numbers of positive results (31 vs. 62; $\chi 2 = 30.031$, $P < 0.001$), however, that were often pathogens likely susceptible to pAP according to Patients´Infection/Colonization History (P.IC.H.) and surveillance Data for Antibiotic Resistance in our Clinic (D.AR.C.).

Overall, pathogens identified by mRT-PCR/bile AND considered resistant to pAP based on P.IC.H and D.AR.C., were likely of clinical relevance. mRT-PCR, as an add-on/complementary assay to CMCs for bile analysis, increases diagnostic sensitivity and may provide valuable input for infection management in selected cCLD-patients undergoing ERCP, thus favoring a more timely and efficient use of antibiotics.

P2.31 Patients with NAFLD exhibit more advanced fibrosis in liver biopsy than patients with other chronic liver diseases

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Introduction Despite extremely high and seemingly rising prevalence of non-alcoholic fatty liver disease (NAFLD), awareness for this health condition is still low. In the present study we analyzed, if this is reflected in clinical routine for advanced diagnostic measures.

Methods Retrospective data of 93 patients with histologically determined fibrosis stage and confirmed etiology was analyzed. Patients were grouped according to chronic liver disease alone (n = 40), concomitant chronic liver disease and NAFLD (n = 29), or NAFLD alone (n = 24). Fibrosis stage and presence of cirrhosis were main outcome measures.

Results Patients with NAFLD were significantly older and had significantly higher body mass index and CAP-values than patients with chronic liver disease. Significantly higher fibrosis stages were observed in patients with NAFLD than in those with chronic liver disease alone (p = 0.003). Presence of cirrhosis was significantly higher in patients with NAFLD than in patients with chronic liver disease (p = 0.01). This was not associated with a significantly different age distribution over fibrosis stages between chronic liver disease and NAFLD. Undergoing liver biopsy 10 years earlier could have possibly prevented progression to cirrhosis in up to 7 patients with NAFLD. This could have potentially saved 35,000 € yearly health care resources.

Conclusion These findings suggest that the time course for development of liver fibrosis and cirrhosis is not fundamentally different between patients with NAFLD or with other chronic liver diseases. Higher rates of cirrhosis observed in patients with NAFLD could potentially be ameliorated by earlier diagnostic work-up and improved monitoring.

P2.32 Benefits of hepatitis B vaccination in patients with chronic liver disease

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Background Hepatitis B vaccine has proven highly successful in preventing hepatitis B virus (HBV) infection and reducing consequential hepatitis B-related disease burden in the countries where vaccination has been implemented. However, few studies have reported the impacts of the hepatitis B vaccination particularly on patients with chronic liver disease (CLD) other than chronic hepatitis B.

Methods To investigate the efficacy of the hepatitis B vaccination on patients with CLD, a large USA cohort of 57306 patients between 2000 and 2020 was obtained through the Observational Health Data Sciences and Informatics (OHDSI) consortium.

Results 2.79 % (1601/57306) of patients with CLD in the USA were (documented to be) vaccinated. Generally, HBV-vaccinated patients with CLD had a significantly better survival (p = 0.000). Patients with liver cirrhosis also showed a significantly improved survival (p = 0.000). By investigating subgroups, particularly patients with chronic hepatitis C (p = 0.000), chronic non-alcoholic liver disease (p = 0.000), or both alcoholic and non-alcoholic-induced cirrhosis (both p = 0.000) all shared significant benefits from HBV vaccination. The observed benefits were independent of the patient´s gender. Notably, vaccination was not demonstrated to be as effective in patients with autoimmune including bile duct-associated liver diseases, Wilson´s disease, or alpha-1 antitrypsin defici-

ency. However, the numbers of enrolled and vaccinated patients in these groups were both low.

Conclusion Our results demonstrated that HBV vaccinated patients with different kinds of CLD generally had significantly better survival. As 97.21 % (55706/57306) of the population were at least documented to be vaccinated, appropriate vaccination status must be clarified for all patients with CLD.

P2.33 Efficient non-invasive detection of liver cirrhosis by LSM

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Background & Aims Early detection of advanced chronic liver disease is critical for patient management and disease surveillance. In routine clinical care it remains challenging to differentiate cirrhosis from lower fibrosis stages without liver biopsy. Aim of the present study was to assess different non-invasive detection tools for separation of cirrhosis from lower fibrosis stages.

Methods Data of 116 patients (51 males, 65 females; mean age 48 ± 16) with chronic liver disease of various etiologies was retrospectively analysed. Liver biopsy was performed between May 2020 and May 2021. Routine laboratory values, serological markers, liver stiffness measurements (LSM) and established non-invasive scores were analyzed for correlations with histological fibrosis stage.

Results Robust and significant correlations with the histological fibrosis stage were identified for LSM (r = 0.65), the FAST score (0.64), the FIB-4 (0.48), serum AST concentration (0.41) NFS (0.33), INR (0.30), LiMAx test results (-0.40), and serum albumin concentration (-0.29). by spearman rank correlation. ROC curves were built for these parameters to separate patients with cirrhosis from those with any other fibrosis stage. The highest AUC was achieved by LSM (0.9130), followed by the FAST score, (0.8842), the FIB-4 (0.8644), the NFS (0.8227), INR (0.8142), serum albumin (0.7710), and serum AST (0.7620). The most promising clinical applicability would be an LSM value of 12.2 kPa achieving 95.7 % sensitivity and 75.3 % specificity.

Conclusions LSM and FAST score seem to be robust non-invasive measurements for liver fibrosis. LSM, FAST score may have potential to reliably detect patients with liver cirrhosis in clinical routine settings.

P2.34 Prediction of liver-related endpoints by non-invasive fibrosis tests – longitudinal follow-up in adults with severe alpha-1 antitrypsin deficiency (Pi * ZZ genotype)

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Aims and objectives Homozygous Pi * Z mutation (Pi * ZZ genotype) confers a strong predisposition to lung and liver disease. Since the pace of liver disease progression and prognostic factors remain unknown, we evaluated risk factors and the predictive utility of non-invasive tests in the European Pi * ZZ liver cohort.

Methods 480 Pi * ZZ subjects without concomitant liver diseases or pathological alcohol consumption received a baseline clinical, laboratory, and elastographic assessment. 407 of them had a detailed follow-up interview at least 12 months after their baseline examination.

Results During a median follow-up of 3.8 years, 25 Pi * ZZ individuals deceased. The main causes of fatality were lung and liver disease, accounting for 9 and 8 deaths, respectively. 18/5 individuals received a lung/liver transplant. 17 Pi * ZZ subjects who developed a hepatic endpoint (liver transplant/death, or decompensated cirrhosis) presented with significantly higher BMI (28 vs. 24 kg/m2, p = .001), liver stiffness measurement (14 vs. 5 kPa, p = 6.0x10-10), AST-to-platelet ratio index (APRI, 1.0 vs. 0.3 units, p = 1.8x10-7), fibrosis-4 index (3.6 vs. 1.3, p = 3.0x10-6), and liver enzymes in their baseline examination. Multivariate Cox regression analysis revealed LSM ≥ 15 kPa (aHR 40.3, 95 % CI 11.0-147.8, p = 2.4x10-8) and APRI ≥ 1.0 units (aHR 37.3, 95 % CI 10.3-135.5, p = 3.9x10-8) as strong predictors of liver-related mortality.

Conclusions LSM and APRI accurately stratify Pi * ZZ individuals according to their risk of liver-related events. Thus, these non-invasive tests may allow risk stratification in clinical practice as well as selection for clinical trials.

P2.35 Role of spleen elastography as surrogate marker for portal hypertension risk stratification in patients with autoimmune liver disease

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Liver stiffness is an important surrogate marker for liver fibrosis and the presence of clinically significant portal hypertension (CSPH). However, a significant impact of pronounced inflammation especially in autoimmune hepatitis (AIH) or cholestasis in primary sclerosing cholangitis (PSC) on liver stiffness values and the risk of false positive results has been demonstrated in the past. We hypothesise that spleen elastography can improve diagnosing CSPH in patients with AILD.

From our tertiary centre 55 patients with AIH, 47 patients with PSC and 87 patients with chronic non-AILD liver diseases were included from a prospectively acquired cohort and liver- and spleen transient elastography and parameters of disease activity and stage were assessed.

Surrogate markers of hepatitis activity or cholestasis correlated positively with liver stiffness in patients with AIH (AST r = 0.338, p = 0.01, and IgG r = 0.418, p = 0.002) and PSC (ALP r = 0.394, p = 0.007). In contrast, spleen stiffness did not correlate with the aforementioned parameters. Along that line, no significant correlation between liver and spleen stiffness was found in patients with AILD (AIH r = 0.172, p = 0.209 ; PSC r = 0.274, p = 0.062), whereas a correlation between spleen stiffness and spleen size can be assumed especially in PSC (r = 0.505, p = 0.001). Spleen stiffness predicted the Baveno-VI criteria for the detection of CSPH in AIH and PSC with an AUC of 0.76 (p = 0.004) and 0.799 (p = 0.001), respectively.

Measuring spleen stiffness may improve the prediction of clinically significant portal hypertension in patients with AILD. Further work is needed to define cut-off values that best predict the presence of CSPH in patients with AILD.

P2.36 Prevalence of metabolic dysfunction-associated fatty liver disease, and its impact on health-related quality of life in people living with HIV

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Background The prevalence of metabolic risk factors and non-alcoholic fatty liver disease (NAFLD) is high among people living with HIV (PLWH). However, the prevalence of metabolic dysfunction-associated fatty liver disease (MAFLD)

and its impact on health-related quality of life (HRQL) in PLWH remains unestablished.

Methods A total of 282 PLWH on ART were prospectively enrolled. MAFLD was defined as the presence of hepatic steatosis and either overweight/obesity, lean/normal weight with additional risk abnormalities or type 2 diabetes. Hepatic steatosis and fibrosis were assessed by vibration-controlled transient elastography (VCTE). The HRQL was measured by the generic EQ-5D-5L questionnaire.

Results The prevalence of MAFLD in the cohort was 26.6 % (n = 75). The median CAP in MAFLD was 320 dB/m. PLWH and MAFLD with type 2 diabetes showed the highest median LSM values (6.3 [IQR 6.2-7.8], p = 0.010). Overall, HIV-related parameters including CD4 count, viral load and treatment regimen were not different between MAFLD and non-MAFLD. PLWH and MAFLD reported significantly lower HRQL (UI-value) compared to non-MAFLD (0.85 ± 0.19 vs. 0.91 ± 0.13, p = 0.008). In a multivariable analysis, MAFLD remained an independent predictor of an impaired HRQL (UI-value) in PLWH (beta = −0.170, 95 CI −0.296, −0.030, p = 0.016).

Conclusion PLWH exhibit a high prevalence of MAFLD and report a significantly lower HRQL compared to PLWH in the absence of MAFLD. Risk-stratification of PLWH according to hepatic steatosis and MAFLD will help to prioritize preventive measures that are likely to support somatic and quality of life aspects.

P2.37 Scheduled Regular Endoscopic Interventions for Patients with Primary Sclerosing Cholangitis Improve Transplant-Free Survival

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Primary sclerosing cholangitis (PSC) is associated with biliary obstructions that can require endoscopic retrograde cholangiopancreatography (ERCP). While beneficial short-term effects of ERCP are well documented, follow-up interventional strategies are less defined and their long-term impact is debated. We retrospectively evaluated the outcome of a scheduled program that has been implemented at our tertiary liver center for over more than 20 years.

Within our program, ERCPs were performed at regular and pre-defined intervals to follow-up or treat previously detected biliary stenosis. We evaluated the outcome of our approach in comparison to patients, receiving endoscopic interventions only on clinical demand. The primary endpoint was transplant-free survival (TFS). The secondary endpoints were occurrence of hepatic decompensation, recurrent cholangitis episodes, development of hepatobiliary malignancies and endoscopy-related adverse events.

We included 268 consecutive patients with diagnosed PSC, 133 receiving scheduled ERCP and 135 receiving on demand ERCP. The rates of TFS since initial diagnosis (median:27 vs. 19 years; p = 0.027) and initial presentation (median:16 vs. 12 years; p = 0.001) were significantly higher in patients receiving scheduled vs. on demand ERCP. Subgroup analysis revealed that progression in cholangiographic findings between the 1stand 2ndERCP (progression cohort) was associated with a poorer outcome compared to patients without progression (non-progression cohort) (median:17 years vs. undefined; p = 0.014).

Our data indicate that implementation of a scheduled ERCP program with interventions performed in predefined intervals improves TFS in patients with PSC. Furthermore, we reveal that progression in cholangiographic findings after the first ERCP procedure enables a prediction of the future disease activity and outcome.

P2.38 The rs72613567:TA polymorphism in HSD17B13 is associated with survival benefit after development of hepatocellular carcinoma

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DOI 10.1055/s-0042-1759967

Background The genetic factors associated with survival following diagnosis of hepatocellular carcinoma (HCC) are not well understood. The goal of this study was to assess if polymorphisms influencing susceptibility to HCC are also associated with HCC prognosis.

Methods United Kingdom Biobank participants diagnosed with HCC after study enrolment were included. The primary outcome event was all-cause mortality. Patients were followed from the date of HCC diagnosis to death or the registry completion date. Five HCC susceptibility polymorphisms were considered: rs738409 (PNPLA3), rs58542926 (TM6SF2); rs72613567 (HSD17B13); rs2242652 (TERT) and rs708113 (WNT3A). Cox regression was used to determine the independent association between these genetic loci and HCC mortality risk. All models included adjustment for age at HCC diagnosis, gender, ethnicity, receipt of curative HCC treatment, severity of underlying liver disease, and liver disease aetiology. Sensitivity analyses were performed using liver-related mortality and HCC mortality as alternative outcome events.

Results The final sample included 439 patients with an average of 1.9 years follow-up per patient. There were 321,295 and 235 all-cause, liver-related and HCC-related deaths. The 3-year all-cause mortality proportion was: 68.8 % (95 %CI: 64.1-73.3). In multivariate analysis, rs72613567:TA (HSD17B13) was the only genetic susceptibility variant associated with liver-related mortality (aHR:0.76; 95 %CI:0.62-0.92; P = 0.005). Other factors associated were Baveno stage 3/4 (aHR:1.65; 95 %CI:1.06-2.59; P = 0.03) and HCC treatment with curative intent (aHR:0.25; 95 %CI: 0.17-0.37; P < 0.001). In sensitivity analysis, rs72613567 remained associated with liver-related (aHR:0.76; P = 0.005); and HCC-related mortality (aHR:0.75; P = 0.013).

Conclusions This study suggests the rs72613567 polymorphism in HSD17B13 influences HCC

Poster Visit Session lll Metabolism (incl. NAFLD) 27/01/2023, 16.20 pm – 16.55 pm

P3.01 Elevated M30 serum levels allow the non-invasive detection of fibrotic NASH in NAFLD with low or intermediate FIB-4

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Background Non-alcoholic steatohepatitis (NASH) and fibrosis are the main prognostic factors in non-alcoholic fatty liver disease (NAFLD). The FIB-4 score has been suggested as initial test for the exclusion of progressed fibrosis. How-

ever, increasing evidence suggests that also NASH patients with earlier fibrosis stages are at risk of disease progression, emphasizing the need for improved non-invasive risk stratification.

Methods Since apoptosis plays an early role for NAFLD progression, we evaluated whether the apoptosis biomarker M30 can identify patients with fibrotic NASH despite low or intermediate FIB-4 values. Serum M30 levels were assessed by ELISA, and FIB-4 was calculated in an exploration (n = 103) and validation (n = 100) cohort of patients with histologically confirmed NAFLD.

Results The majority of patients with low FIB-4 (cut-off value < 1.3) but increased M30 levels (> 200 U/L) had NASH, mostly with histological fibrosis. NASH was also detected in all patients with intermediate FIB-4 (1.3 to 2.67) and elevated M30, from which the majority showed fibrosis. Importantly, in the absence of elevated M30, most patients with FIB-4 < 1.3 and NASH showed no fibrosis.

Conclusion The combination of FIB-4 with M30 enables a more reliable identification of patients at risk for progressed NAFLD and might therefore improve patient stratification.

P3.02 Hepatocyte-specific deletion of group VIA calcium-independent phospholipase A2 leads to protection against MCD diet-induced NASH

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DOI 10.1055/s-0042-1759969

Polymorphisms of phospholipase A2VIA (iPLA2β or PLA2G6) are associated with body weights and blood C-reactive protein. The role of iPLA2β/PLA2G6 in non-alcoholic steatohepatitis (NASH) is still elusive. In GASL2021 abstract, we reported that female mice with myeloid-specific PLA2G6 deletion showed exacerbated hepatic fibrosis after feeding with methionine- and choline-deficient diet (MCDD). Herein, female mice with hepatocyte- (LPla2g6 − / −) specific PLA2G6 deletion were phenotyped after feeding with chow or MCDD for 3.5 weeks. LPla2g6 − / − mice fed with chow displayed an attenuation of blood monocytes and elevation of anti-inflammatory/pro-resolution lipoxin A4 in plasma and liver. Compared with chow, MCDD-fed mutants showed a greater elevation of hepatic lipoxin A4. Consistently, MCDD-fed LPla2g6 − / − mice showed attenuated levels of plasma alanine aminotransferase, plasma non-esterified fatty acids, blood monocytes, and hepatic triglycerides. Hepatic steatosis protection was associated with upregulation of PPARalpha and attenuated hepatic expression of PPARgamma, fatty-acid uptake, triglyceride synthesis, and de novo lipogenesis genes. LPla2g6 − / − mice were also protected against MCDD-induced hepatic fibrosis associated with attenuated expression of IL-6, TNF-alpha, CD115, Collagen1alpha1, Collagen4alpha1, alpha-SMA, TIMP1, TGFbeta-1, vimentin, CHOP, p-JNK, as well as the reduction of sirus-red, alpha-SMA, and cleaved caspase 3 (+) staining. Thus, PLA2G6 inactivation specifically in myeloid cells and hepatocytes led to opposing phenotypes in female mice undergoing NASH. Mechanistically, PLA2G6 inactivation in hepatocytes may induce the mobilization of arachidonate which becomes the substrate of 12/15-lipoxygenases activated by MCDD, thus resulting in the elevation of protective lipoxin A4. Hepatocyte-specific PLA2G6 inhibitors may be further developed for treatment of this disease.

P3.03 Fatty liver disease in PCOS: metabolic, genetic and hormonal phenotypes of two independent European cohorts

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Background Fatty liver disease (FLD) is common in women with polycystic ovary syndrome (PCOS). In this study, we genotype risk factors for FLD and phenotype liver status in females with PCOS coming from Germany and Poland

Patients Women with PCOS and controls were prospectively recruited at two university centres in Germany and Poland (Homburg and Katowice). Liver stiffness measurements (LSM), controlled attenuation parameters (CAP) and non-invasive HSI, FLI, FIB-4 scores were determined to assess hepatic fibrosis and steatosis. Alcohol abuse was regarded as an exclusion criterium. Five genetic variants associated with FLD were genotyped using TaqMan assays

Results We recruited 42 German (age range 18–53 years), 143 Polish women with PCOS (age range 18–40 years) and 534 controls. Compared with Polish patients, Germans were older, presented with more severe metabolic profiles and had significantly higher LSM (median 5.9 kPa versus 3.8 kPa). In the German cohort, carriers of the PNPLA3 p.I148M risk variant had an increased LSM ($P = 0.01$ in a multivariate model). In the Polish cohort, no effects of PNPLA3 were detected ($P > 0.05$) but the FLD-protective MARC1 allele was linked with significantly lower serum aminotransferase activities. The HSD17B13 polymorphism was associated with lower concentrations of 17-OH progesterone, total testosterone, and androstenedione ($P < 0.05$).

Conclusions Liver injury is common in females with PCOS and seems modulated by genetic risk factors. In particular, genetic variants associated with FLD might modify the progression of liver disease in women with PCOS.

P3.04 Die Rolle von MARC1 bei chronischen Lebererkrankungen in humanen und murinen Modellsystemen

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Hintergrund MARC1 wird mit Lebererkrankungen in Verbindung gebracht, da die genetische Variante p.A165T im MARC1-Gen mit niedrigen Blutcholesterinspiegeln und Leberenzymwerten, sowie einer Verringerung des Schwergrades der nicht-alkoholischen Fettlebererkrankung assoziiert ist. Ziel dieser Studie war die Untersuchung von MARC1 und anderen assoziierten Genen bei verschiedenen Lebererkrankungen in murinen und humanen Modellsystemen.

Methodik Als Mausmodelle dienten sowohl Abcg5/g8-defiziente Tiere, bei denen der Transporter selektiv in der Leber oder im Dünndarm ausgeschaltet wurde (Fettleber-Modell, Chow-Diät, bzw. 12-wöchige Fütterung mit einer lithogenen Diät), sowie Abcb4-defiziente Tiere verschiedenen Alters (Fibrose/Zirrhose-Modell, unbehandelt, bzw. für 48h behandelt mit Diethylnitrosamin (DEN)). In allen Tieren wurde die Expression der Gene Marc1, Hsd17b13, Gckr, Trib1 und ApoE untersucht. Außerdem wurden Patienten mit PBC, bzw. PSC auf die genetische Variante p.A165T im MARC1-Gen untersucht.

Ergebnisse Die Genexpressionsanalysen zeigten eine signifikante Reduktion in der Expression von Marc1, sowohl im Fibrose- als auch im Fettlebermodell. Außerdem wurde Hsd17b13 in den darmspezifischen Abcg5/g8-defizienten Tieren unter lithogener Diät verstärkt exprimiert, wohingegen ApoE in den hepatischen Abcg5/g8-defizienten Tieren vermindert exprimiert wurde. Im Fibrosemodell zeigte sich ebenfalls eine verminderte Expression von ApoE Die Western-Bot-Analyse bestätigte im Wesentlichen die Expressionsergebnisse. Bei den PSC-, bzw. PBC-Patienten konnte keine Assoziation des SNPs mit der Erkrankung gezeigt werden.

Schlussfolgerungen Die Ergebnisse zu Marc1 in murinen Modellsystemen zeigen, dass Marc1 eine Rolle sowohl bei der Fettlebererkrankung als auch bei der Fibrose spielt, wobei eine Assoziation der genetischen Variante im Marc1-Gen bei PSC- und PBC-Patienten nicht bestätigt werden konnte. Deshalb sind weitere Studien notwendig, um die Rolle von Marc1 bei chronischen Lebererkankungen aufzuklären.

P3.05 Religious fasting: effects on metabolism and liver steatosis in type 2 diabetes patients

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Background Religious fasting during the holy month of Ramadan requires abstaining from food and liquids. It is allowed to consume food and drinks from dusk till dawn. In the summer month, fasting can be regarded as a modified form of intermittent fasting, resulting in reduced and timed calorie intake. Type 2 diabetes (TD2) is a risk factor for developing non-alcoholic steatohepatitis (NASH). Several studies suggested some benefits of interval fasting for TD2 and NASH patients. Here we aimed to characterize the impact of a one-month fasting period on glucose and lipid metabolism liver health in a cohort of T2D patients.

Methods 20 patients were included. In addition to anthropometric measurements, the liver's status was assessed by Fibroscan®, including the controlled attenuation parameter (CAP) measurement. Further, patients' blood samples were collected at the beginning and end of the four-week fasting period to quantify critical parameters of liver injury and lipid and glucose metabolism.

Results Liver stiffness and CAP values showed modest changes following the fasting period. The patients experienced significant weight loss at the end of the fasting month. LFTs, apoptosis marker (M30), and adiponectin significantly decreased after the fasting period. Serum levels of triglycerides were lower after following the interval fasting period. Glucose levels did not change significantly, but serum levels of C-peptide and insulin increased following the fasting period.

Conclusion In this cohort of T2D patients, we demonstrated that a 4-week intermittent fasting period resulted in an improvement in different serum parameters associated with glucose, liver, and lipid metabolism.

P3.06 Tick-tock – Circadian regulation of liver metabolism represented in a kinetic model

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The circadian rhythm is a decisive regulator for metabolic homeostasis especially in the liver. The importance of diurnal control is highlighted by the increased risk of liver diseases, obesity and metabolic syndrome due to disturbance of circadian rhythms. However, time resolved in vivo studies of liver metabolism are rare and molecular resolved, kinetic models can be used for metabolic phenotyping based on proteomic data, enabling linking circadian rhythmicity of protein abundances to metabolic regulation.

We investigated the rhythmicity of liver metabolism in male C57BL/6N mice using kinetic models for liver samples isolated every three hours. Additionally, we correlated plasma metabolite profiles with metabolic liver functions.

Our analysis revealed a rhythm of 12 hours for lipid metabolism, ethanol detoxification and partly carbohydrate metabolism in the liver. However, gluconeogenetic capacity, fructose and urea synthesis capacity were obviously not underlying circadian regulation. We could show a correlation between plasma fatty acid concentrations and fatty acid liver metabolism. Concerning detoxification capacities, ethanol utilization capacity was highly associated with plasma glucose concentrations, but no significant correlations with plasma metabolites could be found for urea synthesis and ammonia uptake capacities.

The model helps to better understand whether circadian rhythms are intrinsic and independent of nutrient availability or follow diurnal dietary patterns. By accounting for the circadian regulatory properties of all enzymes, our model integrates the accumulated knowledge from decades of biochemical research and allows quantitative predictions of system behavior as a function of circadian rhythmicity.

P3.07 Assessment of liver steatosis and liver damage in patients with ulcerative colitis

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Introduction Recent data indicate that hepatic steatosis is higher in patients with inflammatory bowel disease (IBD), including ulcerative colitis (UC). We have recently shown that TNFα treatment is associated with lower rates of steatosis in patients with Crohn's disease compared to controls. This could be related to a reduction of IBD-specific risk factors like intestinal inflammation. This study aimed to examine the hepatic status in patients with UC compared to patients with IBS who lack intestinal inflammation.

Methods This cross-sectional study evaluated patients with established UC (n = 23) and IBS (n = 8), analyzing serum markers of liver injury, measurement of transient elastography (TE), and controlled attenuation parameter (CAP). In addition, we assessed features of cell death and adipokines by ELISA measurement in the serum.

Results The analysis included 31 patients (15 male, 16 female; age 50,86 (23-78)) with UC (74,2 %) or IBS (25,8 %). Six patients with UC received anti-TNFα and 16 therapy with aminosalicylic acid. Mean liver stiffness measured by TE and M30 were significantly higher in patients with UC than in patients with IBS. M30 and M65 showed a positive association with ASAT, ALAT, and triglycerides in patients with UC but not in patients with IBS.

Discussion Liver stiffness and M30 indicate higher hepatic inflammatory response and apoptosis in patients with ulcerative colitis than IBS. As epithelial apoptosis is well described in UC, impact of M30 measurement is limited.

P3.08 Biomarkers, Imaging, and Safety in a Well-compensated NASH Cirrhotic Cohort Treated With Resmetirom, a Thyroid Hormone Receptor-beta Selective Agonist, for 52 Weeks

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MAESTRO-NAFLD-1 is a randomized, double-blind, placebo-controlled Phase 3 trial evaluating resmetirom, a thyroid hormone receptor-beta selective agonist, in NASH patients identified using noninvasive biomarkers and imaging (NCT04197479). MAESTRO-NAFLD-1 includes an open-label resmetirom arm in well-compensated NASH cirrhotic patients. Eligibility required ≥ 3 metabolic risk factors and NASH cirrhosis (diagnosed by biopsy or accepted criteria). Primary and key secondary endpoints include safety, relative percent reduction in MRI-PDFF (Week 16), LDL-C, apoB, and triglycerides (Week 24), and fibrosis markers. All patients received 80 or 100mg resmetirom daily for 52 weeks. Cohort 1 (n = 105) has completed 52 weeks of treatment. Cirrhosis stage inversely correlated with baseline MRI-PDFF. At Week 52, resmetirom reduced FibroScan CAP by 42dB/m ($p < 0.0001$) and FibroScan VCTE (LSM) by 7.6kPa ($p = 0.02$). In patients with baseline MRI-PDFF > 5 % (5 % = ULN), resmetirom reduced MRI-PDFF by 37 % ($p < 0.0057$). At Week 52, resmetirom reduced MRE by 0.68kPa; 34 % of patients achieved an MRE reduction ≥ 15 %. GGT and ALP were reduced with resmetirom (by 27 % and 18 %, respectively; $p = 0.04$ for both). Liver volume, which was elevated at baseline, was reduced by 15.9 % at Week 16 ($p < 0.0001$), independent of baseline MRI-PDFF. Liver volume reduction correlated with reduction in MRE, MRI-PDFF, TIMP, P3NP, and SHBG. Resmetirom reduced LDL-C (20 %), apoB (20 %), triglycerides (21 %), and Lp(a) (30 %), independent of cirrhosis stage. Resmetirom was well tolerated. BP decreased by 4-5mmHg. Overall, resmetirom reduced markers of cardiovascular risk and fibrosis in NASH cirrhotic patients.

P3.09 Augmenter of liver regeneration (ALR) alters iron homeostasis regulating proteins by modifying the IL-6-STAT3-pathway

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Augmenter of liver injury (ALR), an anti-apoptotic, anti-oxidative and anti-inflammatory protein is widely expressed and known for its hepatotropic properties. Interleukin 6 (IL-6) is a pro-inflammatory cytokine and the main trigger of the acute-phase response (APR) following tissue damage, infection or inflammation. Both ALR and IL-6 have been described to play pivotal roles in the process of liver damage and its consecutive regeneration. The acute-phase-protein hepcidin is the principal regulator of systemic iron homeostasis, decreasing iron-uptake and lowering plasma iron concentration. Hepcidin mRNA expression is mainly activated by IL-6. Furthermore, iron accumulation and lower hepcidin expression was observed in alcohol-fed ALR-deficient mice. We have shown earlier, that ALR has a dual effect on IL-6-signaling/APR in hepatocytes dependent of its localization. Exogenous ALR (rALR) attenuates and endogenously ALR enhances IL-6-signaling. Therefore we analyzed the impact of ALR on proteins responsible for regulating iron homeostasis. HepG2 cells (wt and sfALR-overexpressing) cells were treated with either IL-6 or BMP6, w/o rALR, and proteins of iron metabolism were analyzed qRT-PCR and western blot. Treatment with rALR diminished IL-6 induced expression of TfR1 and Zip14 (both iron uptake) in wt and sfALR-HepG2. Furthermore, rALR decreased hepcidin upon IL-6 stimulation only in wt cells. IL-6 induced expression of iron exporter ferroportin (FPN1) is reduced by rALR in sfALR-HepG2 cells. Additionally, rALR modulating effects on BMP6 induced expression was seen in sfALR-HepG2 for FPN1 and TfR1. Application of rALR might ameliorate IL-6 induced iron overload regulation of iron homeostasis by reducing iron uptake and increasing iron export.

P3.10 Intestinal BMP-9 expression correlates with FGF19 in a cohort of adipose patients

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Bone morphogenetic protein (BMP)-9, a member of the TGFβ-family, is believed to be mainly produced in the liver. Serum levels of BMP-9 were reported to be reduced in newly diagnosed diabetic patients and BMP-9 overexpression ameliorated steatosis in the high fat diet-induced mouse model of obesity. Because both, FGF21 and 19 were also described to protect the liver from steatosis, we investigated if such functions of BMP-9 might be related to a regulation of FGF's using samples from a cohort of adipose patients with or without diabetes (n = 11 non-diabetic, 16 diabetic, all: BMI > 40).

First, we analyzed BMP-9 protein levels in serum and found no reduction in the sub-group of diabetic patients. Nevertheless, hepatic BMP-9 mRNA negatively correlated with steatosis (NAS score) especially in those patients that did not (yet) develop diabetes. Furthermore, hepatic BMP-9 expression also negatively correlated with serum LPS levels. In situ hybridizations revealed that BMP-9 is expressed not only in the liver but also in the small intestine. Interestingly intestinal – but not hepatic – BMP-9 mRNA levels negatively correlated with diabetes and positively correlated with intestinal FGF19 (but not FGF21) expression.

Our data imply that especially intestinal and not only hepatic BMP-9 associates with diabetes and steatosis development and controls intestinal FGF19 expression. The data support the conclusion that increased levels of BMP-9 would most likely be beneficial under pre-steatotic conditions, making supplementation of BMP-9 an interesting new approach for future therapies aiming at prevention of the development of a metabolic syndrome and liver steatosis.

P3.11 In vitro modulation of hedgehog signaling in primary hepatocytes

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The liver coordinates a variety of pathways and is responsible for lipid, glucose and xenobiotic metabolism. Hedgehog (Hh) signaling is involved in organ homeostasis and regulation of metabolism. When dysregulated, it contributes to various liver diseases such as NAFLD. Hepatocytes, which comprise the parenchyma and therefore the majority of hepatic cells, are a target for therapeutic approaches. As in vitro manipulation of Hh is a part of preclinical trials, our study aims to provide comprehensive data of the effects of assorted modulators on Hh and the interacting Wnt pathway in hepatocytes.

Five Hh inhibitors (Cyclopamine, RU-SKI43, GANT61, Vismodegib, Budesonide) and two Hh activators (Triamcinolone acetonide, SAG) were assessed in primary

murine and human hepatocytes to analyze their effects on gene expression (RNA sequencing and qPCR) and metabolic effects (Seahorse analyzer assays). The data show that only two of the compounds modulate Hh according to their known function as inhibitors (Cyclopamine, GANT61), while promoting Wnt signaling. They also impact lipid metabolism which is congruent with data from transgenic mice with Hh inactivation in hepatocytes. The remaining modulators do not show postulated regulation of Hh in hepatocytes and conflicting results for Wnt signaling and lipid metabolism.
From these results we conclude that while Hh modulation in primary hepatocytes is possible, many compounds have opposing effects on Hh compared to reports in literature as well as effects on a variety of other pathways. This underlines the importance of determining suitability and specificity of a compound for varying cell types for in vitro experiments.

P3.12 Infectious mononucleosis is associated with an increased incidence of non-alcoholic fatty liver disease (NAFLD): A cohort study of 27,718 patients

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Background Non-alcoholic fatty liver disease (NAFLD) has become the leading cause of liver cirrhosis and hepatocellular carcinoma worldwide. Although various genetic and lifestyle-related risk factors have been identified, its exact pathophysiology has not yet been unraveled fully. While acute EBV infection in the setting of infectious mononucleosis can lead to acute hepatitis, the long-term hepatic sequelae of infectious mononucleosis are still poorly understood.
Methods This retrospective cohort study included 13,859 patients with and 13,859 matched individuals without a diagnosis of infectious mononucleosis from the Disease Analyzer database (IQVIA) to evaluate the association between infectious mononucleosis and NAFLD.
Results Within 10 years of the index date, 2.64 % of patients with infectious mononucleosis and 1.78 % of individuals without infectious mononucleosis had been diagnosed with NAFLD ($p < 0.001$). The incidence of NAFLD was 263.9 and 164.5 cases per 100,000 person-years among individuals with or without infectious mononucleosis. Multivariable regression analyses indicated that infectious mononucleosis was significantly associated with the incidence of NAFLD (HR: 1.73, 95 %CI: 1.38–2.17, $p < 0.001$) both among women (HR: 1.73, 95 %CI: 1.27–2.35) and men (HR: 1.70, 95 %CI: 1.21–2.40). The association between infectious mononucleosis and NAFLD was most pronounced for the groups aged between 41 and 50 years (HR: 2.94, 95 %CI: 1.69–5.11) and > 50 years (HR: 2.68, 95 %CI: 1.44–3.58).
Conclusion Infectious mononucleosis was found to be significantly associated with the incidence of NAFLD in a large cohort from Germany. These findings could stimulate research efforts to better understand the currently unknown pathophysiology of this emerging global medical burden.

P3.13 Changes in the Etiology of Chronic Liver Disease by Referral to a Fibroscan Center: Rising Prevalence of the Non-Alcoholic Fatty Liver Disease

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Background and Aim Chronic liver disease (CLD) is a leading cause of morbidity and mortality worldwide with wide etiological spectrum. FibroScan is used for follow-up of fibrosis and steatosis. This single center study aims to review the distribution of indications by referral to FibroScan.
Materials and Methods Demographic characteristics, CLD etiologies, and FibroScan parameters of the patients, who were referred to our tertiary care center between 2013 and 2021 were retrospectively evaluated.
Results Out of 9,345 patients, 4,946 (52.9 %) were male, and median age was 48 [18-88]. Non-alcoholic fatty liver disease (NAFLD) was the most common indication (N = 4,768, 51.02 %) followed by hepatitis B (N = 3,194, 34.18 %) and hepatitis C (N = 707, 7.57 %). Adjusting for age, sex, CLD etiology, the results revealed that patients with older age (OR = 2.908, CI: 2.597-3.256, $P < 0.001$), and patients with hepatitis C (OR = 2.582; CI = 2.168-3.075; $P < 0.001$, ALD OR = 2.019; CI = 1.524-2.674, $P < 0.001$), AIH (OR = 2.138; CI = 1.360-3.660, $P < 0.001$) had increased odds of advanced liver fibrosis compared to NAFLD.
Conclusion NAFLD was the most common indication for referral to FibroScan. Further studies are warranted to explore the etiological spectrum of CLD in Turkey. TASL

P3.14 Effect of a one-month consumption of different alcohol-free beer beverages on metabolism and liver health.

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Introduction Whether regular moderate alcohol consumption does pose a severely increased risk to a healthy person is still heavily discussed. However, gender-specific limits for daily alcohol consumption have been lowered recently. The positive effect of alcohol-free beer drinks as alternatives to classic beer has not yet been proven. In this monocentric, randomized, multi-arm study, we investigated the influence of various non-alcoholic beer beverages [pilsner (PI), wheat beer (WB), mixed drink/Radler (MD), and water (WA)] as control, on fatty degeneration and damage to the liver, glucose and lipid metabolism.
Methods 36 healthy young male probands were included. Blood samples were collected at the beginning and end of a four-week period in which they had to consume two bottles (á 330ml) of the respective beverage daily. The status of the liver was additionally examined by transient elastography, including measurement of the controlled attenuation parameter (CAP).
Results Triglyceride levels increased in all beer groups compared to the WA group. However, in the PI group, the increase lacked significance. HDL levels were also higher in the WA and PI groups. Deterioration of markers of glucose metabolism was detected in all groups compared to controls (including insulin, fasting glucose levels, C-Peptide, and HbA1c). ALT and AST showed a significant increase in the MD group, while transient elastography results showed no differences.
Discussion In this cohort of young healthy me, we demonstrated that four weeks of alcohol-free beverage consumption deteriorated different serum parameters associated with glucose-, liver-, and lipid- metabolism.

P3.15 Morbidly obese NAFLD patients scheduled for bariatric surgery present with a milder disease phenotype and different distribution of adipose tissue as compared to obese NAFLD patients: analysis of a large real-world cohort

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DOI 10.1055/s-0042-1759982

Background Up to 90 % of morbidly obese patients have NAFLD, but it is not clear if these patients present a milder NAFLD phenotype. Aim of this study is to analyze the phenotype of NAFLD in patients with and without (w/o) bariatric surgery (BarSur).

Method 814 patients were prospectively included in a single-center study. NAFLD was diagnosed either by CAP/ultrasound (n = 410) or histologically (n = 348). 513 patients were genotyped for PNPLA3 p.I148M variant.

Results 510 NAFLD patients with and 249 w/o BarSur were included in this study. Case-control matching for age, sex, and T2DM was performed (n = 225 per group). NAFLD BarSur patients had more often arterial hypertension and hypertriglyceridemia compared to NAFLD w/o BarSur. However, NAFLD BarSur patients had significantly lower ALT (33 vs. 56U/l), AST (27 vs. 39U/l), gGT, ferritin and FIB-4 (0.87 vs. 1.24). Additional matching for p.I148M showed no different finding. No difference was observed between historical and preoperative ALT ruling out any effect of conditional phase in NAFLD BarSur. After case-control matching in histologically diagnosed patients (n = 82 per group) NAFLD w/o BarSur had significantly higher frequency of NASH (74 % vs. 52 %), steatosis, ballooning and fibrosis (F2-F4: 44 % vs. 7 %). Visceral/ subcutaneous adipose tissue (AT) ratio assessed by abdominal CT-scan (n = 78) was significantly higher in NAFLD w/o BarSur reflecting a different distribution of AT.

Conclusion NAFLD patients with BarSur present a clinically milder disease phenotype in terms of serum profile and histology in our large patient cohort. A different distribution of AT might be an underlying pathophysiological condition.

P3.16 Incidence of hepatic adenomas in adult glycogen storage disease type Ia/b

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Introduction Patients with glycogen storage disease type Ia/b frequently suffer from benign hepatic tumours. The confounding factors for hepatic adenomatosis in this disease are obscure and its potential to malignant transformation into HCC is not known. Some centres have reported HCC rates of up to 12 % in GSD patients.

Methods Single-center retrospective study in 37 adult GSD type Ia/b patients from 2013-2022.

Results Out of 37 screened patients with secured diagnosis of GSD type Ia or Ib, a series of 10 patients (4 males, 6 females) with hepatic adenomas were identified. The number of adenomas ranged from 1 to > 10, with an average size of 30 ± 15,7 mm at time of Dx. The mean age at diagnosis was 27 years (range 19-38). Average transient elastography (TE) in these patients was 4,9 + /- 0,5 kPa, which was not different from the control group (4,9 + /- 4 kPa). Alpha-fetoprotein (AFP) was normal in all patients. In 6 patients the size of the lesion was increasing (mean growth 19 mm), in 2 patients it was stable and in 2 patients it was decreasing. In 3 patients the imaging features or size of the lesion led to surgical resection, but no HCC was diagnosed.

Conclusion GSD type Ia/b patients with diagnosed hepatic adenomas need close clinical surveillance. Evidence-based guidelines weighing ultrasound and elastography against MR-based techniques in conjunction with established markers have to be developed. Influence of therapy and the modifying role of empagliflozin have to be investigated in randomized clinical trials.

P3.17 Dynamics of liver injury and regeneration in a mouse model of bacterial infection related acute-on-chronic liver injury (BI-ACLI)

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Background and aim Acute-on-chronic liver failure (ACLF) is defined as acute decompensation of chronic liver disease in the presence of an acute trigger like bacterial infection (BI). We aimed to establish a mouse model, mimicking BI-ACLF conditions to study physiological processes during disease onset and progression.

Methods C57BL/6J (n = 16; wild-type, WT) and Abcb4-/- (n = 16; knock-out, KO) mice were intraperitoneally injected either with 0.9 % NaCl or 4 mg/kg LPS to establish four groups: control (WT + NaCl), acute (WT + LPS), chronic (KO + NaCl) and acute-on-chronic group (KO + LPS). RNA sequencing was used to identify differentially expressed genes (DEGs). DEGs were confirmed by qRT-PCR and immunoblot.

Results IFN-γ (stimulation of M1-polarization) and CD64, CD86 and CCR7 (M1 markers) were upregulated in the KO-LPS group only. M2 markers remained unchanged (CD206 and Arg1) or downregulated (CD163). IL-17 and IL-22 are known to be regulated differentially in response to IL-6 and TGF-β levels, i.e., IL-6 alone induces T cells (TH17 cells) to produce IL-22, whereas the combination of both IL-6 and TGF-β results in the production of IL-17 but not IL-22. In line with this tissue response to liver injury, we observed high-levels of IL-6 and IL-22 in KO-LPS group, but IL-17 and TGF-β did not differ.

Conclusions M1/M2 imbalance in our model implies active disease progression, since M2 macrophages are involved in resolution of inflammation and regeneration. On the other hand, high levels of IL-22, which promotes tissue protection and regeneration, suggest an ongoing protective tissue response. Further research is required to delineate these dynamic pathophysiological processes.

P3.18 Identification of alcohol induced-differential gene expressions in retinol metabolism using transcriptomics

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Background and Aims Alterations in retinol metabolism due to alcohol may have pathophysiological effects in alcohol-associated liver disease (AALD). Using a transcriptomics approach, we aimed to identify alcohol induced-differential gene expression involved in retinol metabolism in wild-type (WT, acute model) and knock-out (KO) mice with a chronic liver injury (acute-on-chronic injury). Materials and

Methods Total RNA was extracted from WT and Abcb4-/- (KO) mice, which were either fed control (WT/Cont and KO/Cont) or ethanol diet, followed by an acute ethanol binge (WT/EtOH and KO/EtOH; n = 3/group). Transcriptome analysis was performed by RNA sequencing of mRNA libraries constructed from liver samples using NextSeq500 (Illumina). Gene expression data was evaluated using pathways annotated by Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/).

Results A total of 21 and 14 genes involved in retinol pathway were upregulated in chronic plus binge alcohol-induced WT and KO models, respectively. Among them, retinoid catabolizing cytochrome P450 (CYP) enzyme genes, Cyp26a1, Cyp26b1, Cyp26c1, Cyp2c29, Cyp2b10, Cyp2a5, and Cyp4a14 were common DEGs identified in WT and KO models. However, Cyp2c55, Cyp3a11, and Cyp4a10 were present only in KO group, suggesting that bile acid toxicity present in KO mice was the major driver of altered expression.

Conclusions Significant alterations in expression of CYP450 genes after ethanol exposure in both groups demonstrate that our preclinical model represents a valuable tool for investigating the retinol metabolism in the pathogenesis of AALD. Subsequent studies are required to provide further insights into the

underlying mechanisms and the progression of these alcohol-induced liver injury.

P3.19 Role of the membrane-bound bile acid receptor (TGR5) in the alteration of macrophage metabolism during bacterial infections

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TGR5 (Gpbar-1) is a membrane-bound bile acid receptor considered a metabolic regulator involved in BA synthesis and glucose metabolism. The activation of TGR5 in macrophages is associated with a reduced inflammatory response. Krüppel-like factors (KLFs) play critical roles in the transcriptional regulation of pathways involved in the metabolism of glucose and lipids and the immune response. The present study aims to determine the contribution of TGR5 to metabolism in macrophages during bacterial infection.

TGR5 knockout (KO) and wildtype (WT) mice were injected with Listeria monocytogenes (L.m.). Flow cytometry has been used to examine the glucose and lipids uptake in macrophages. The RNA-seq approach investigated the differential expression of transcripts involved in metabolism in infected macrophages.

TGR5 and KLF5 mRNA expression was up-regulated, and KLF4 expression was down-regulated in BMDMs and livers from WT mice after L.m. infection. Furthermore, TGR5 KO mice were more susceptible to L.m. infection with increased mortality rates, elevated inflammatory cytokines, and BA levels. The uptake of glucose and cholesterol was altered in TGR5 KO macrophages. The RNA-seq analysis indicated 58 up-regulated transcripts in glucose metabolism and 32 down-regulated transcripts in cholesterol metabolism in WT macrophages compared to TGR5 KO after infection.

The changes in the differential expression of transcripts involved in the glucose and cholesterol metabolism in WT macrophages, together with the up-regulation of TGR5 and KLF5 and down-regulation of KLF4 in these cells and livers of WT animals after Listeria infection, indicate the vital role of TGR5 for proper immune and metabolic functions.

P3.20 Different impact of the ketoses fructose and allulose on hepatocyte glucose metabolism

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The rare sugar allulose is a stereoisomer of fructose. In vivo studies indicated that allulose is absorbed in the intestine and transiently accumulates in the liver. However, it is not metabolized but excreted in the urine. In addition, in vivo data suggested that allulose may enhance glucose storage in hepatic glycogen and reduce blood glucose concentration. To further explore the mechanisms underlying these in vivo observations, the direct impact of allulose in comparison with fructose on glucose and glycogen metabolism was studied in isolated rat hepatocytes in culture.

In absence of insulin, fructose enhanced whereas allulose inhibited the incorporation of 14C-glucose into glycogen. Insulin stimulated the incorporation of 14C-glucose into glycogen. The insulin-dependent increase was blunted by allulose and allulose reduced the glycogen content in hepatocytes incubated in presence of insulin for 24 h. In absence of insulin, hepatocytes released glucose into the medium. The release was enhanced by fructose. By contrast, allulose reduced this glucose release. Insulin shifted the glucose balance towards a net uptake of glucose into the hepatocyte. While the insulin-dependent uptake was not affected by a low (3 mM) concentration of allulose, it was impaired by a high concentration (10 mM) of allulose. At neither concentration allulose interfered with the insulin-dependent Akt phosphorylation.

At variance with in vivo observations, allulose reduced glucose incorporation into glycogen in isolated hepatocytes but nevertheless reduced glucose output from hepatocytes in absence of insulin.

P3.21 Development Of Metabolic Disorders In Liver Transplant Recipients

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Background The development of metabolic abnormalities following liver transplantation (LT) represents an important disease burden in liver transplant recipients The aim of this study was to assess the prevalence of metabolic abnormalities in liver transplant recipients.

Methods A total of 114 adult liver transplant recipients with a minimum followup of 1 year were enrolled into the analysis. Demographics and laboratory data were collected from patients' charts. The median follow-up period after LT was 6 years (range, 1-22 years).

Results Mean age was 56.0 ± 11.5 and 63 % were male. Living donor liver transplantation was performed on 88 patients (77 %). There were fourty-six smokers (40 %). Hepatitis B virus infection (41, 36 %) was the main indication to LT. The majority of the patients (101,89 %) were on tacrolimus-based treatment. Median follow-up period after LT was 7.5 year (range 1-20 year). In post-transplant period body mass index (BMI) was 27.8kg/m2, 29.78 % of them were obese. Thirty-two patients (28 %) developed new-onset diabetes mellitus (NODAT), 60.1 % hypertension, 40.7 % hyperlipidemia, and 51.3 % metabolic syndrome (Table 1). The mean post transplant HbA1C was 6.22 ± 1.57. The post-transplant median atherosclerotic cardiovascular disease (ASCVD) risk score was 16.5. Sixteen patients (14 %) had a history of a cardiac event post-transplant.

Pre-transplant hypertension, diabetes, antihypertensive and antidiabetic drug use, ASCVD score and BMI were found to be higher in those with post-transplant metabolic syndrome (Table 2).

Conclusion Metabolic disorders are frequent complications after liver transplantation.

P3.22 Effect Of Prognostic Noninvasive Tests On Disease Outcome Of Alcohol-Related Liver Disease

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Aims The aims of the study were to determine the characteristics of patients with alcohol-related liver disease (ALD) and to identify whether the factors affect the course of disease.

Methods Between 01/2001–12/2021, 187 patients, who were diagnosed with ALD were included. FIB-4, NFS, Forns, APRI and AST/ALT were calculated. Data were collected from outpatient visit charts.

Results All 187 patients were Caucasian, mean age was 51,6 ± 10,1 years, and gender was predominantly male (98 %). At diagnosis, 59 patients had hepatosteatosis/steatohepatitis and 128 patients had cirrhosis, 43,8 % of them were decompensated: 15 % of the patients were classified as having Child-Pugh A, 56 % Child-Pugh B, 29 % Child-Pugh C. Median MELD score was 14 (range: 7–33). Ascites (71 %) was the most common causes of decompensation, followed by variceal bleeding (18 %), and hepatic encephalopathy (11 %). Ten patients (8 %) were diagnosed with HCC. The mean FIB-4, NFS, Forns, APRI and AST/ALT were 3,22 ± 2,99 (median: 2,28), −0,86 ± 2,9 (median: −1,26), 5,95 ± 2,6 (median: 5,8), 1,17 ± 1,34 (median: 0,71) and 1,51 ± 1,0 (median 1,2) respectively. The

scores of noninvasive tests were significantly higher in cirrhotic patients (p > 0,001). During a median follow-up of 32.6 months, cirrhosis was developed in 20 % of noncirrhotic patients, decompensation was developed in 79 % of compensated cirrhotic patients. HCC was developed in 7 cirrhotic patients. Twenty-one patients (11 %) underwent transplantation, and 72 (39 %) patients died. The FIB-4, NFS, Forns and APRI were significantly higher in transplanted and non-survival patients (p < 0,05). Conclusion; ALD has high liver-related morbidity and mortality. Non-invasive tests may provide disease outcome. TASL

P3.23 A Combination Of Non-Invasive Tests In Detection Of NAFLD-Related Advanced Fibrosis Is Not Superior To MRE Alone

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Background and Aims The superiority of combination of non-invasive tests for assessment of liver fibrosis in patients with non-alcoholic fatty liver disease (NAFLD) is unclear. The aims of the present study were to investigate the superiority of combination of magnetic resonance elastography (MRE) and transient elastography (TE) or MRE and FIB-4 index or MRE alone in the detection of advanced fibrosis in patients with NAFLD.

Methods 119 consecutive patients with NAFLD were included into analysis. Histological samples were interpreted using NASH CRN Scoring system. Liver stiffness was measured by vibration-controlled TE using a FibroScan probe (Echosens, Paris, France) and MRE with a 1.5-T MR system. Advanced fibrosis was defined as ≥ stage 2 fibrosis. The cut-off points for MRE, TE and FIB-4 index for advanced fibrosis were 3.2 kPa, 9.0 kPa and 1.6, respectively.

Results The mean age of patients was 53 years. Sixty-five patients (55 %) diagnosed with stage 2 fibrosis. The median interval between MRE and liver biopsy was 30 days. MRE, TE and FIB-4 index demonstrated a significant accuracy for the detection of significant fibrosis (p = < 0.001, 0.014 and 0.014, respectively). MRE, TE and FIB-4 index demonstrated significant accuracy with an AUROC of 0.848 ± 0.036 (p < 0.001), 0.632 ± 0.052 (p = 0.012) and 0.664 ± 0.051 (p = 0.001), respectively. Diagnostic performance of MRE was superior than TE (p < 0.001) and FIB-4 index (p = 0.001). A combination MRE and TE or MRE and FIB-4 index were not superior than MRE alone (p = 0.880 and p = 0.45, respectively).

Conclusion MRE alone is accurately used for non-invasive

P3.24 Impairment of patient-reported outcomes among patients with nonalcoholic fatty liver disease: a registry-based study

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DOI 10.1055/s-0042-1759991

Background Patients with NAFLD tend to have more impairment in their health-related quality of life and other patient-reported outcomes (PROs) with more advanced fibrosis.

Aim To assess the association of PROs with select non-invasive tests (NITs) for fibrosis including FAST, Agile 3 + , and Agile 4 scores.

Methods Patients with an established diagnosis of NAFLD seen in a tertiary care clinic in Istanbul, Turkey, were enrolled in the NAFLD/NASH Registry. The FAST, Agile 3 + , and Agile 4 scores were calculated using liver stiffness measurements by transient elastography and laboratory parameters. PROs were assessed using FACIT-F, CLDQ-NASH, and WPAI instruments (total 17 domain and summary scores).

Results There were 1509 patients with NAFLD (mean age: 49 ± 11 years, 50 % men, 41 % employed, 30 % advanced fibrosis, 20 % cirrhosis). The mean FAST, Agile 3 + , and Agile 4 scores were 0.39 ± 0.26, 0.35 ± 0.31, and 0.12 ± 0.23, respectively. Subjects with lower FAST, Agile 3 + , and Agile 4 + scores had the highest scores in select domains of FACIT-F, CLDQ-NASH, and WPAI (p < 0.05 in comparison to subjects with elevated or high-risk NIT scores). Correlations with continuous NITs were significantly negative for Emotional and Functional well-being (FACIT-F), Activity/energy, Systemic symptoms, Worry, and total scores (CLDQ-NASH), and Activity of WPAI (p < 0.05); the strongest for Worry (CLDQ-NASH) with FAST (R = -0.17, p < 0.0001).

Conclusion Patients with NAFLD and high FAST, Agile 3 + , or Agile 4 scores experience impairment of health-related quality of life. TASL

P3.25 PNPLA3 I148M polymorphism aggravate metabolic liver disease under Long-term high fat diet

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Background Nonalcoholic fatty liver disease (NAFLD), a widespread disease, is associated with multiple genetic risk factors. The PNPLA3 I148M polymorphism of the phospholipase patatin-like phospholipid domain containing protein 3 (PNPLA3) has been shown to worsen disease progression during metabolic liver disease. Current PNPLA3 I148M knockin animals fail to recapitulate the human phenotype.

Methods Mice bearing wild-type Pnpla3 (Pnpla3WT), or the human polymorphism PNPLA3 I148M (Pnpla3I148M) were subjected to a HFD for 24 and 52 weeks. A detailed analysis including the basic phenotype, inflammation, proliferation, cell death, fibrosis progression, microbiota as well bile acid (BA) metabolism was performed.

Results Only under long-term high fat diet and the existence of obesity, Pnpla3I148M animals developed fatty liver disease and liver injury associated with more immune cells infiltration compared to Pnpla3WT animals. Apoptosis and proliferation were increased in Pnpla3I148M mice. Moreover, Pnpla3I148M mice with HFD for 52 weeks have stronger fibrosis and hepatic stellate cells activation as well as increased ductular proliferation and higher BA stool levels. Bacterial dysbiosis during HFD feeding were influenced by HFD feeding (36 %) and the PNPLA3 I148M genotype (12 %). RNA-sequencing analysis of liver tissue defined a Pnpla3I148M specific expression pattern, which defines Kupffer cell and monocytes-derived macrophages as significant drivers of disease progression in Pnpla3I148M animals.

Conclusion The PNPLA3 I148M knockin model only develops a severe NASH liver phenotype only after long-term feeding leading to obesity. Here significant differences were found compared to Pnpla3WT animals showing that Pnpla3I148M animals develop a specific phenotype triggered by bacterial dysbiosis and higher BA levels associated with a stronger Kupffer cell- and monocytes-derived inflammatory response leading to stronger fibrosis progression.

P3.26 Sex differences regarding the effect of dietary vitamin E on the liver and metabolic profile.

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Background and Aims Non-alcoholic fatty liver disease (NAFLD) is characterised by lipid droplet accumulation in the hepatocytes and affects approximately 20-30 % of the general population. Currently, there are no approved pharmacological agents available to treat NAFLD. There is growing evidence

supporting the potential benefits of Vitamin E in reducing steatosis and lobular inflammation in non-diabetic patients with NAFLD. Despite numerous trials conducted to assess the efficacy of vitamin E supplementation in patients with NAFLD, there is a lack of understanding of its preventative effects.

Method We analyzed the UK Biobank dataset and selected our study population based on the availability of nutritional assessment. We investigated the association between dietary vitamin E intake and various disease phenotypes and serum metabolites, as well as serum vitamin E levels and NAFLD prevalence.

Results Data from > 210,000 participants demonstrate that increased dietary vitamin E intake was associated with a lower prevalence of NAFLD and overall mortality. Each standard deviation (SD) vitamin E increase per kJ energy intake reduced the association of NAFLD ICD-10 diagnosis (K76.0) by 12.5 % (p

P3.27 Effects of transcription factor ChREBP on NAFLD-driven Hepatocarcinogenesis

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Background and Aims The transcription factor carbohydrate response element binding protein (ChREBP) is involved in the modulation of glycolytic and lipogenic pathways [1]. Dysregulation of lipogenesis facilitates the development of non-alcoholic fatty liver disease (NAFLD) [2]. Previous studies have suggested that ChREBP is associated to development of hepatocellular carcinoma (HCC) [3]. However, the molecular pathogenesis of ChREBP-related hepatocarcinogesis in response to the prolonged high fat diet (HFD) feeding in the mouse model remains unexplored.

Methods Male C57BL/6J (WT) and Liver-ChREBP-KO (L-KO) mice were maintained on either a HFD (46 % fat; n = 153) or a control diet (10 % fat; n = 45) for 3, 6 and 12 months to induce metabolic syndrome. Body weight and blood glucose levels were measured once in a month. After perfusion, formalin fixation and paraffine embedding was performed. The liver sections were then examined by histological and immunohistochemical analysis.

Results We have investigated the consequences of hepatic ChREBP deficiency on steatosis, inflammation, fibrosis and glycogen accumulation in the liver. HFD fed mice showed significant increase ($p < 0.05$) in body weight from 5-months onwards compared to the control. HFD feeding also caused a significant increase ($p < 0.05$) in liver weight and blood glucose levels in 12-months mice. Proliferative activity (Ki-67 index) of hepatocytes showed higher ($p < 0.05$) in HFD than control in 12-Month mice. WT HFD fed mice showed higher lipid content in the liver compared to the L-KO mice. There is a progressive increment of lipid accumulation in hepatocytes, indicating that 6-months mice showed 20-30 % fat accumulation, which gradually increased to 80 -100 % fat in 12-months group. After 12-months, HCC development was only evident in WT mice (4/195 animals).

Conclusion Our present study implicates that prolonged HFD feeding contributes to the development of NAFLD, which in turn progresses to HCC. Furthermore, after liver ChREBP deletion, HCC development is absent, suggesting an oncogenic role of ChREBP in hepatocarcinogenesis [1–3].

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Poster Visit Session IV Tumors
28/01/2023, 08.30 am – 09.05 am

P4.01 Phenotypic characterization of colorectal liver metastases: capsule versus no capsule and the potential role of epithelial mesenchymal transition

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DOI 10.1055/s-0042-1759995

Background Colorectal liver metastases (CRLM) can be encased in a fibrous capsule separating cancer from normal liver tissue, which correlates with increased patient survival. This study elucidates cellular and molecular mechanisms of capsule formation and the possible role of epithelial mesenchymal transition (EMT).

Methods 34 CRLM from 34 patients were analyzed by EMT-pathway-profiling- and custom-PCR-arrays to identify differences in gene expression between CRLM with (n = 20) and without capsule (n = 14). In parallel, those 34 CRLM were used to analyze 16 gene products at the metastasis margin via immunohistochemistry.

Results Encapsulated CRLM show elevated expression of signal transduction pathways and effector molecules involved in EMT; E-Cadherin and Keratin-19 were more prevalent and transcription as well as translation (immunohistochemistry) of pGSK-3-β, SOX10, Tomoregulin-1 and Caldesmon were increased. By contrast, loss of E-cadherin and the prevalence of snail 1 were increased in CRLM without capsule. Collagen I, III and Versican were identified as capsule components with the extracellular matrix fibers running concentrically encircling the malignant tissue and parallel to the invasive front. Caldesmon was also demonstrated as capsule constituent.

Conclusions The fibrous capsule of CRLM is produced by cells with mesenchymal characteristics; it functions as a protective border by both, features of fiber architecture and inhibition of invasive growth through EMT recruiting mesenchymal cells such as myofibroblasts by transformation of surrounding epithelial or even carcinoma cells. By contrast, EMT demonstrated in non-encapsulated CRLM may lead to a more mesenchymal, mobile and tissue destructive carcinoma cell phenotype and facilitate malignant spread.

P4.02 N-cadherin as a diagnostic marker to discriminate primary from metastatic liver carcinomas

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The differential diagnosis between the primary liver cancer hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA) from liver metastases is of crucial therapeutic importance, but the histopathologic diagnosis may be challenging. We previously identified the adherens junction glycoproteins N- and E-cadherin as hallmarks of normal hepatocytes, cholangiocytes

and derived tumors. In line with literature, we showed that N-cadherin is restricted to neural and mesenchymal cells, whereas epithelia are vastly negative, with the exception of liver carcinoma and carcinoma during the phenomenon of epithelial to mesenchymal transition. Therefore, we hypothesized that N-cadherin may distinguish between carcinoma derived from the liver versus other origin. During this study, we evaluated E- and N-cadherin in 2,489 different tumors using immunohistochemistry, and compared our results with previously published 882 cases of primary liver cancers including 570 HCCs and 312 iCCAs. Most carcinomas irrespective of their origin showed strong positivity for E-cadherin. A strong staining reaction with antibodies against N-cadherin was present in HCC and iCCA. With the exception of clear cell renal cell carcinoma (23.6 % of cases), N-cadherin was rarely expressed in adenocarcinomas of the gastrointestinal tract (0-0.5 %), lung (7.1 %), pancreas (3.9 %), gynecological organs (0-7.4 %) and breast (2.2 %) nor urothelial (9.4 %) or squamous cell carcinoma (0-5.6 %). Furthermore, N-cadherin was frequently detected in tumors of endocrine origin such as thyroid cancer (29.2 %), neuroendocrine tumors (25-75 %), as well as in malignant melanoma (46.2 %) and malignant mesothelioma (41 %). In conclusion, N-cadherin is a useful marker for the distinction of primary versus metastatic liver carcinoma ($p < 0.01$).

P4.03 Identification and validation of a plasticity driver of Combined Hepatocellular-Cholangiocarcinoma using functional interspecies comparison

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Primary liver cancer mainly comprises hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA), which may occur together as combined HCC-CCA (cHCC-CCA). Lineage-tracing experiments demonstrated CCA formation from hepatocytes as well as HCC originating from ductular cells in mice highlighting the plasticity of epithelial liver cells.

Comparative exome and transcriptome sequencing of both components of cHCC-CCA revealed 54 differentially expressed and/or mutated candidate genes between both tumor components. Their potential plasticity driving function was validated by in vivo RNAi screening using transposon-based mosaic mouse models for HCC and intrahepatic CCA development, respectively. After tumor development, livers were harvested, individual tumor nodules were dissected and histologically analyzed regarding a potential phenotypic switch, which was validated by immunohistological detection of hepatocellular (HNF-4alpha) and biliary (keratin 19) markers.

Thrombospondin 3 (THBS3), which was initially found to be mutated in the HCC compartment of human cHCC-CCA, was identified and validated as a phenotypic driver. Both, knockdown of THBS3 and expression of the synonymous mutation resulted in a cHCC-CCA phenotype in the kras-mutated, p19-deficient murine CCA model induced by hydrodynamic tail vein injection.

Functional analyses were conducted in vitro in isogenic cell lines derived from our mouse models. Overexpression of mutant THBS3 increased AFP and decreased SOX9 mRNA expression in CCA cells, while overexpression of the wildtype protein decreased albumin expression in HCC cells. The phenotype induced by overexpression of mutant THBS3 in CCA cells was phenocopied following shRNA-mediated knockdown of THBS3 in cells, confirming that THBS3 acts as a driver of cellular plasticity in liver cancer.

P4.04 The long non-coding RNAs lnc-ADM-2 and lnc-NBPF3-9 represent potential novel targets in hepatocellular carcinoma

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Introduction Long non-coding RNAs (lncRNAs) are > 200 nucleotide long RNA molecules operating on epigenetic, transcriptional and post-transcriptional levels to regulate cancer-related mechanisms like proliferation, pluripotency and apoptosis. However, expression and function of most lncRNAs – and their roles in hepatocellular carcinoma (HCC) – is widely unknown. The aim of this study was to identify and functionally verify novel lncRNAs in HCC.

Methods The expression of 87 potentially cancer-associated lncRNAs was analyzed using a qRT-PCR-based lncRNA expression array. Expression profiles of HCC cells (Hep3B, PLC) were compared to primary human hepatocytes (PHH) and activated hepatic stellate cells (aHSC). Sanger sequencing and DNA gel electrophoresis confirmed the identified lncRNAs. SiRNA-pool-mediated knockdown and subsequent boyden chamber and anchorage-dependent clonogenicity assays were used to study the lncRNA-mediated effects on migration, invasion and stem cell properties.

Results Differential regulation of eight lncRNAs in HCC cells was identified. However, only two lncRNAs (lncADM-2, lncNBPF3-9) were specifically and strongly overexpressed in HCC cells compared to both hepatocytes and aHSCs, indicating cancer-specific de-regulation. DNA gel electrophoresis and sanger sequencing confirmed strong expression of the predicted lncADM-2 transcript and revealed differential splicing of lncNBPF3-9 in HCC cells. First functional experiments showed decreased migration and clonogenicity after knockdown of lncADM-2.

Conclusions The study revealed overexpression of two novel lncRNAs (lncADM-2, lncNBPF3-9) in HCC. Splicing of a predicted intron of lncNBPF3-9 did not occur in HCC cells resulting in a longer, potentially functional novel transcript variant. Moreover, lncADM-2 functionally promotes migration and clonogenicity in HCC, outlining a potential novel therapeutic target.

P4.05 Understanding CEACAM6 mechanisms as a key player in aggressive behaviour of gallbladder cancer

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DOI 10.1055/s-0042-1759999

Introduction Gallbladder cancer (GBC) is an aggressive malignancy and represents the most common biliary tract cancer (BTC). Molecular drivers and biomarkers for GBC are poorly identified. This study combines proteomic analysis of patient samples, in vitro characterizations, and molecular mechanism investigations of potential oncogene in GBC aggressiveness.

Methods Mass-spectrometric analysis of 5 GBC and 5 healthy gallbladder FFPE tissues were performed. Differentially expressed proteins were analysed to elucidate oncogenic protein in GBC. Transient siRNA knockdown and stable inducible cells were established to understand the proteins' molecular function and subjected to RNASeq analysis to unravel the significantly perturbed pa-

thways. BirA-BioID followed by mass-spectrometry was conducted to identify protein-protein interaction partners.

Results Carcinoembryonic Antigen-related Cell Adhesion Molecule 6 (CEACAM6) presented as one of the strongest upregulated proteins in GBC proteomics (fold-change = 5.54, adj.pval ≤ 0.01). CEACAM6 overexpression promoted migration and invasion of GBC cells, while CEACAM6 knockdown reduced cell proliferation, colony formation, and migration. Integrin and AKT pathways were among the most significant enriched pathways in RNASeq analysis of CEACAM6 overexpressing cells. Integrin alpha-2 (ITGA2) and integrin beta-1 (ITGB1) were found as interacting partners of CEACAM6 based on BirA-BioID and were confirmed using proximity ligation assay. AKT activation and its downstream signalling pathways were inhibited by CEACAM6 knockdown and AKT inhibitors, and thus reduced GBC aggressiveness.

Conclusion CEACAM6 supports GBC aggressiveness by increasing migration, invasion and clonogenicity. In addition, CEACAM6 interacts with ITGA2 and ITGB1, and inhibition of CEACAM6 and/or administration of AKT inhibitors might be potential strategies to abrogate GBC aggressiveness.

P4.06 Investigating the SLIT-ROBO signaling pathway in cholangiocarcinoma

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Introduction Cholangiocarcinoma(CCA) is a rare but highly aggressive malignancy of the bile duct with inadequate therapy options and poor prognosis. A recent study identified genetic alterations in SLIT/ROBO pathway exclusively in the invasive form of CCA. The SLIT/ROBO pathway acts as axon-guidance-cues during neuronal development, it additionally orchestrates multiple processes in organ development, like cellular growth, attachment, and motility. The aim of this project is to functionally characterize the SLIT/ROBO signaling pathway in CCA.

Methods ROBO1 and ROBO2 receptors were introduced into intrahepatic-CCA cells with a doxycycline-inducible expression system by using lentiviral transduction. By using small-interferenceRNA SLIT1,2,3 were knocked down. Functional analyses were applied to assess the role of ROBO receptors and SLIT ligands in viability, migration, wound healing and colony formation. RNA-seq analysis was performed to detect downstream effects of ROBO signaling.

Results Upon doxycycline-induced overexpression of ROBO1 and ROBO2 receptors, migration ability of RBE intrahepatic-CCA cell line was decreased. Colony formation assays resulted in decreased colony size and area. In contrast ROBO receptors did not have significant effects on CCA cell viability. In addition, knockdown of SLIT2 resulted in increased migration and colony area which is consistent with the observed inhibitory role of ROBO receptors. RNA-seq analysis revealed multiple pathways involving in migration and cancer to be regulated by ROBO receptors.

Conclusion SLIT/ROBO signaling suppressed migration and clonogenicity of cholangiocarcinoma cells suggesting a tumor suppressive role in CCA. Further experiments will reveal interaction partners of the receptors and crosstalk between signaling pathways involving in SLIT/ROBO mediated migration suppression.

P4.07 Integrated genotype-phenotype analysis of Familial adenomatous polyposis-associated hepatocellular adenomas

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DOI 10.1055/s-0042-1760001

Background & Aims Familial adenomatous polyposis (FAP) is an autosomal dominant syndrome caused by a germline mutation in the adenomatous polyposis coli (APC) gene, characterized by numerous colorectal adenomas. In addition, the patients may develop extraintestinal manifestations. Several cases of hepatocellular adenomas (HCA) detected accidentally in FAP-patients have raised the question whether they represent a specific manifestation of FAP or a mere coincidence.

Methods Analysis of all resected liver lesions of FAP patients (1991-2021) of the Institute of Pathology of Heidelberg University resulted in the detection of 5 hepatocellular adenomas in 3 patients. Comprehensive morphological, immunohistological and molecular analyses were employed, including targeted next-generation sequencing.

Results The analyzed HCAs showed no cytological or histological atypia. Immunohistochemically, a diffuse, strong positivity for glutamine synthetase was found in the absence of a nuclear beta-catenin staining. In two patients, the adenomas showed a moderate immunoreactivity against serum amyloid A. Consistent with the diagnosis of FAP, molecular profiling revealed a pathogenic germline mutation of the APC gene in all analyzed adenomas as well as deleterious somatic second hits. All somatic mutations localized between codons 1345 and 1577. No mutations were found in beta-catenin encoding Catenin Beta 1.

Conclusions HCA in FAP patients is a specific, although rare neoplastic manifestation of this inborn disease and represents a distinct group of liver cell adenomas. These benign tumors represent an important differential diagnosis to hepatic metastases in FAP patients and require adequate clinical and molecular (diagnostic) assessment for optimal patient guidance.

P4.08 Minichromosome maintenance (MCM) protein complex is upregulated in hepatocytes of Schistosoma mansoni-infected hamsters

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Questions Schistosomiasis is one of the most common parasitic infections of humans worldwide. The eggs of Schistosoma mansoni induce chronic liver inflammation and are involved in hepatic carcinogenesis, especially hepatocellular carcinoma HCC. The minichromosome maintenance protein (MCM) complex is a helicase controling cell-cycle DNA replication. MCM expression is highly increased in HCC patients. Therefore, we analysed whether the MCM complex is also upregulated during S. mansoni infection.

Methods Hamsters (n = 5) were infected either with both genders (bisex, bs; producing eggs) or unisexual S. mansoni (single sex, ss; no eggs produced) or non-infected (control). Liver tissue of these hamsters was investigated by western blotting and IHC for expression of the MCM subunits 4, 6, and 7. Furthermore, HepG2 cells were treated with soluble egg antigen (SEA) and analysed for MCM-mRNA by qRT-PCR.

Results The expression levels of MCM4/6/7-mRNAs were significantly increased in livers of bs-infected hamsters (containing eggs) compared to ss- or non-infected hamsters (no eggs), suggesting the involvement of schistosome eggs. IHC staining in liver tissue of the bs-group revealed MCM6 nuclear accumulation in hepatocytes surrounding the granulomas. Moreover, in vitro treatment of HepG2 cells with SEA led to an increase of MCM6-mRNA.

Conclusion Recently, S. mansoni infection has been discussed as a predisposition for HCC. Our data support this hypothesis by a hamster infection model. We demonstrate that S. mansoni infection, especially schistosome eggs, disturb replication licensing by upregulating MCM protein complexes, thus facilitating carcinogenesis.

P4.09 Intratumoral heterogeneity in human hepatocellular carcinoma – a protein-based approach

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Background Comprehensive molecular analysis of human hepatocellular carcinoma (HCC) revealed different molecular subclasses, but its translation to the proteomic level remains elusive.

Methods 58 HCC patients were analyzed by multispectral imaging using tissue microarrays to enable parallel detection of four proteins at a single tissue section. Protein expression datasets were analysed using a machine learning-based algorithm (uniform manifold approximation and projection), followed by cluster and spatial analysis (sparse non-negative matrix factorization) to visualize the distribution of molecular subclasses within individual HCC nodules.

Results Unbiased analysis at single-cell level revealed three different clusters: cluster A, defined by high phospho-ribosomal protein S6 kinase and C-reactive protein; cluster B, characterized by diffuse upregulation of glutamine-synthetase; and cluster C showing overexpression of the progenitor cell marker EpCam. However, 55 % of the tumors could be assigned to a particular molecular cluster. However, 45 % of cases showed the presence of several molecular clusters within individual cases, thus highlighting the proteomic diversity of human HCC at the single-cell level and the presence of intratumoral heterogeneity in many human HCCs.

Conclusion We established a protein-based subclassification of HCC, which like the TCGA classifier identified three different subsets of human HCC. The presence of intratumoral heterogeneity in about half of the cases analyzed suggests that a precision oncology approach is likely to select for the outgrowth of tumor cell clusters not targeted by the specific targeted approach. Consequently, identifying cases with high intratumoral heterogeneity may allow for a more precise stratification of patients to different treatment options.

P4.10 Schistosoma mansoni verstärkt die hepatozelluläre Proliferation bei Diethylnitrosamin-induzierter Karzinogenese

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Einleitung Schistosomiasis ist eine der häufigsten parasitären Infektionskrankheiten weltweit. Aufgrund klinischer Fallreporte und Tierstudien wird angenommen, dass S. mansoni die hepatozelluläre Karzinogenese verstärken könnte. Ziel dieser Studie ist es, die Interaktion des Parasiten mit dem Wirtsparenchym vor diesem Hintergrund zu klären.

Methoden Unser Tiermodell umfasst vier Gruppen männlicher C57BL/6-Mäuse: Acht Tiere wurden mittels Badeinfektion mit S. mansoni infiziert, 12 erhielten eine Injektion mit dem Hepatokarzinogen Diethylnitrosamin (DEN), 12 Mäuse wurden sowohl mit S. mansoni infiziert als auch mit DEN behandelt. Zudem umfasst eine Kontrollgruppe sechs unbehandelte Tiere. Mittels qRT-PCR, Western Blot, Immunhistochemie, KOH-Verdau, Proteome Profiler und verschiedenen ELISAs wurden die neun Wochen nach Infektion entnommenen Lebern auf Leberzellschädigung, Eilast, Inflammation, oxidativen Stress, Proliferation, Fibrose sowie Marker onkogener Signalwege hin analysiert.

Ergebnisse Western Blot Analysen aller Mäuse zeigten eine verstärkte Expression der Proliferationsmarker MCM2 und PCNA in den Leberlysaten S. mansoni-infizierter Tiere. Darüber hinaus korreliert die Expression der analysierten Proliferationsmarker stark mit der individuellen Eilast in den Lebern. Bei hoher Eilast zeigten die Tiere, die zudem eine DEN-Injektion erhalten hatten, eine noch höhere Replikationsrate, was mittels Immunhistochemie bestätigt wurde. Soluble egg Antigene aus S. mansoni Eiern stimulieren die Expression der Proliferationsmarker auch in primären murinen Hepatozyten.

Schlussfolgerung Die vorliegenden Ergebnisse belegen die Induktion des Zellzyklus der Hepatozyten im Rahmen einer Infektion mit S. mansoni in vivo und in Zellkultur. Diese Zellproliferation wird durch gleichzeitige Exposition der Tiere gegenüber einem etablierten Hepatokarzinogen gesteigert. Unsere Befunde bieten eine mögliche molekularbiologische Erklärung für eine durch S. mansoni verstärkte hepatozelluläre Dysplasie.

P4.11 Impact of viral aetiology in the Phase 3 HIMALAYA study of tremelimumab plus durvalumab in unresectable hepatocellular carcinoma (uHCC)

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DOI 10.1055/s-0042-1760005

Background In HIMALAYA (NCT03298451), a single, high priming dose of tremelimumab plus durvalumab (STRIDE) significantly improved overall survival (OS) vs sorafenib, and durvalumab was noninferior to sorafenib in uHCC (Abou-Alfa et al. 2022). Viral aetiology is associated with hepatic impairment in HCC development and may influence immunotherapy activity. Thus, we analysed the impact of viral aetiology on clinical outcomes.

Methods This exploratory analysis assessed STRIDE, durvalumab and sorafenib in patients with HBV, HCV or nonviral/other (NV) aetiology. OS hazard ratios (HRs) were calculated using a Cox proportional hazards model. A post hoc multivariate analysis was used to identify chance imbalances in key prognostic factors that may bias estimated treatment effects.

Results and Conclusions Baseline characteristics were similar across treatment arms in the HBV and NV subsets. In the HCV group, multivariate analysis identified imbalances in two prognostic variables: extrahepatic spread (EHS) and ALBI. OS and progression-free survival were improved with STRIDE vs sorafenib in the HBV and NV groups, but not in the HCV group . Using a stratified Cox proportional hazards model to account for imbalances in EHS and ALBI in the HCV subset, OS HRs favoured STRIDE vs sorafenib. Results for durvalumab vs sorafenib showed similar trends to those for STRIDE vs sorafenib .
These results confirm the benefits of STRIDE and durvalumab in patients with uHCC, irrespective of underlying aetiology.

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P4.12 A novel approach of combining BH3-mimetics induces apoptotic cell death in Hepatocellular Carcinoma (HCC) cells

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DOI 10.1055/s-0042-1760006

Introduction Prognosis for advanced-staged HCC is still poor and therefore it is essential to develop new therapeutic approaches. Our focus is on a new treatment concept consisting of BH3-mimetic combinations. BH3-mimetics are inhibitory compounds, which bind pro-survival-proteins of the BCL-2-family and thus can induce apoptosis.

Aims & Methods HCC cell lines Hep3B, HepG2 and Huh7 were treated with MIK665 and ABT199 (Venetoclax), inhibiting Mcl-1 and Bcl-2. Cells were stimulated separately or with a combination of both drugs. Flow cytometry was used to analyse cell death induction. Caspase activity was measured via luminescence-based assays. Cleavage of caspases and PARP was determined by Western blot.

Results Single drug administration of 5µM ABT199 or 6µM MIK665 resulted in up to 9 % cell death induction after 48 h. The combination of both drugs resulted in up to 85 % cell death after 48h, suggesting a synergistic effect of both drugs. The most pronounced effect was detected in HepG2. An increased caspase 9 activity up to 54 % and an increased caspase 3/7 activity up to 470 % could be measured after 4h of combination treatment. Furthermore, we observed a time- and dose-dependent increase of caspase 3/7 activity. Concordantly, combined drug administration resulted in cleavage of caspases 9 and 3 and PARP-1, indicating an efficient induction of apoptosis.

Conclusion The combination of two different BH3-mimetics is effective in inducing cell death via an apoptotic pathway in HCC cell lines. These findings now offer a variety of options to explore the efficacy of various BH3-mimetic combinations in HCC.

P4.13 Clinical characterization of HCC/CCC mixed cancers in a population-based cohort

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DOI 10.1055/s-0042-1760007

Diagnosis of hepatocelluar-carcinoma favors imaging modalities and biopsy is performed in only a minority of patients. Tumors displaying partially characteristics of cholangiocarcinoma (CCA) and being characterized as mixed HCC/CCA tumors may not be identified. However, these tumors may benefit from alternative treatment strategies. The aim of our study was to characterize the clinical course of HCC/CCA cancers to evaluate the need of distinct treatment options.

This population-based cohort study investigated 8221 patients diagnosed in Baden-Wuerttemberg between 2009 and 2019. 5973 HCC cases, 2092 CCA tumors and 156 mixed HCC/CCA. Details regarding sex, age, histologic type, stage, and grade were registered, as well as survival.

1.9 % of patients were diagnosed with HCC/CCA cancers. 62.8 % of those mixed HCC/CCA patients were older than 65 years. This was comparable to HCC but more than among CCA patients. 54 % of mixed HCC/CCA patients were diagnosed as stage IV, which is more than for HCC (33.3 %) but less compared to CCA (68.5 %). Most patients were diagnosed with grade 3 HCC/CCA (56.9 %).

Overall median survival of HCC/CCA patients was significantly worse compared to HCC (11.1 vs. 15.5 months, p = 0.001) and rather comparable to CCA (11.3 months). To confirm predictors of survival, the significant parameters were further examined by a multivariate Cox regression analysis among patients with complete data sets. Further subgroup analyses did not demonstrate gender differences.

Data from our study demonstrate that HCC/CCA tumors appear to have worse overall survival than HCC. Therefore, diagnosis by histology is important to provide them with accurate treatment options.

P4.14 Outcomes by baseline liver function in patients with unresectable hepatocellular carcinoma (uHCC) treated with tremelimumab and durvalumab in the Phase 3 HIMALAYA study

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Background In HIMALAYA (NCT03298451), a single, high priming dose of tremelimumab + durvalumab (STRIDE) significantly improved overall survival (OS) vs sorafenib and durvalumab was noninferior to sorafenib (Abou-Alfa et al. 2022) in uHCC. Liver function is frequently impaired in HCC patients and it is important to evaluate efficacy and safety of systemic therapies for these patients.

Methods HIMALAYA included patients with Child-Pugh Score class A. Baseline liver function was evaluated using albumin-bilirubin (ALBI) score. Exploratory analyses assessed OS, objective response rate (ORR), and safety in patients classified into ALBI grade 1 and ALBI grade 2/3 subgroups.

Results & Conclusions Baseline characteristics in the subgroups were similar across treatment arms. In ALBI grade 1 patients, mOS (95 %CI) was 23.43 months (19.19-28.75) with STRIDE, 21.16 months (17.38-25.86) with durvalumab, and 19.02 months (15.67-23.16) with sorafenib; 36-month OS rates were 38.0 %, 27.0 %, and 27.3 %, respectively.

ORRs were 21.7 % for STRIDE, 18.7 % for durvalumab, and 7.4 % for sorafenib. In ALBI grade 2/3 patients, mOS (95 %CI) was 11.30 months (9.33-14.19) with STRIDE, 12.29 months (9.30-16.03) with durvalumab, and 9.72 months (7.23-11.76) with sorafenib; 36-month OS rates were 21.8 %, 22.5 %, and 12.9 %, respectively. ORRs were 18.3 % for STRIDE, 15.2 % for durvalumab, and 2.7 % for sorafenib. Safety in the ALBI subgroups was generally consistent with the full analysis set.

These results support the use of the STRIDE (T300 + D) regimen as a new treatment option in patients with uHCC, regardless of baseline ALBI grade.

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P4.15 Genetically determined expression of lipoprotein lipase is linked to hepatocellular carcinoma in alcohol-associated cirrhosis

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Background Progression of alcohol-associated liver disease (ALD) is driven by genetic predisposition. The rs13702 variant in the lipoproteinlipase (LPL) gene is linked to non-alcoholic fatty liver disease, but LPL is not expressed in healthy liver. We aimed at clarifying its role in ALD.

Methods Patients with alcohol-associated cirrhosis, with (n = 385) and without hepatocellular carcinoma (HCC) (n = 656), with HCC due to viral hepatitis C (n = 280), controls with alcohol abuse but without liver damage (n = 366) and healthy controls (n = 277) were genotyped for the LPL rs13702 polymorphism. LPL mRNA expression in human liver specimen and in liver cell lines was investigated. Results Frequency of the LPL rs13702 CC genotype was lower in ALD patients with HCC in comparison to ALD patients without HCC both in the initial and the validation cohort (3.9 % vs. 9.3 % and 4.7 % vs. 9.5 %; $p < 0.05$ each), compared to patients with viral HCC (11.4 %), alcohol misuse without cirrhosis (8.7 %) or healthy controls (9.0 %). This protective effect (OR = 0.5) was confirmed in multivariate analysis including age (OR = 1.1/year), male sex (OR = 3.0), diabetes (OR = 1.8), and carriage of the PNPLA3 I148M risk variant (OR = 2.0). Similar effects were seen on population level in the UK Biobank cohort. Liver expression of LPL mRNA was dependent on LPL rs13702 genotype and significantly higher in patients with alcohol-associated cirrhosis compared to controls and alcohol-associated HCC. While hepatocyte cell lines showed negligible LPL expression, hepatic stellate cells expressed LPL, with increase after pioglitazone stimulation.

Conclusion The LPL rs13702 CC genotype confers protection against HCC in ALD.

P4.16 The role of the pancreatic polypeptide-neuropeptide Y receptor 4-axis in hepatocellular carcinoma progression

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Introduction Pancreatic polypeptide (PP) is secreted by PP cells in the endocrine pancreas and is known to affect the liver's glycogen storage. PP acts as a specific ligand of the G protein-coupled transmembrane receptor (GPCR) Neuropeptide Y receptor type 4 (Y4R). GPCRs play crucial roles in the development and progression of liver cirrhosis. However, the potential role of the PP-Y4R-axis in most types of cancer including hepatocellular carcinoma remains completely unclear and was addressed in this study.

Methods Primary human hepatocytes (PHH) and HCC cell lines (PLC, Hep3B, HepG2) were used for functional and expression analysis. Patient derived samples of HCC and corresponding non-tumorous liver tissues were used for in vivo validation of gene expression. For the specific knockdown of PP and Y4R, siRNA-Pools were used. Quantitative RT-PCR and Western blot analysis were performed for quantification of mRNA and protein levels, respectively. Real-time cell analysis and Clonogenicity assays were used to investigate proliferation and stem cell properties. Boyden chamber migration assays were applied for migration analysis.

Results Y4R and PP expression levels were strongly upregulated in HCC in vitro and in patient tissues. PP-mediated Y4R-stimulation induced proliferation, ERK-activation, clonogenicity and chemotactic migration in HCC cells.

Conclusions The PP-Y4R-axis represents a promising novel target in HCC which will be investigated in more detail in ongoing studies.

P4.17 A synthetic lethal strategy to target chromosome 8p deleted cancers

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In the past years the concept of synthetic lethality, where inhibition of certain gene products can act as specific vulnerabilities for cancer cells with a particular genetic makeup, was proposed to guide new targeted therapies. With this concept in mind, we aimed to identify a specific target for chromosome 8p deletions.

Using large-scale functional-genomic screening data of over 527 well-characterized cancer cell lines, we were able to identify a dependency of chromosome 8p deleted cancer cell lines on Mitoferrin-2 (MFRN2) a mitochondrial iron transporter. Interestingly, we found that Mitoferrin-1 (MFRN1), the paralog of MFRN2, resides on chromosome 8p and is frequently deleted in liver cancer. We found a strong correlation between the cellular dependency on MFRN2 and the MFRN1 expression levels, possibly explaining why MFRN2 is a synthetic lethal target for 8p deletions. Genetic epistasis experiments in human liver cancer cell lines revealed that knockout of MFRN2 alone was accompanied by reduced cell growth only in cell lines with low MFRN1 expression, whereas combined knockout of MFRN2 or MFRN1 led to reduced cell growth irrespective of the MFRN1 levels. Moreover, exogenous re-expression of MFRN1 in cell lines with low - or no endogenous MFRN1 expression rescued the observed phenotype upon MFRN2 knockout. Strikingly, targeting MFRN2 in human HCC xenograft models led to complete tumor regression in MFRN1-depleted cancer cells. Finally, human tissue samples provided evidence that MFRN2 could be a therapeutic target for a synthetic lethal directed therapy in a subset of primary HCC patients with low MFRN1 expression.

P4.18 Genotype-to-phenotype mapping in mouse models of liver cancer

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Intratumoral genetic heterogeneity and tumor cell-stroma interactions are emerging topics in cancer research. The lack of experimental strategies has so far precluded systematic analyses of how heterogenous complex genetic alterations affect the tumor microenvironment in a native context. We attempt to overcome this limitation by combining spatial omics readouts with in vivo functional genomics tools to install and identify combinatorial genetic perturbations in an autochthonous mouse model of liver cancer.

We leverage hydrodynamic tail vein injection (HTVI) to stably deliver genetic material capable of gene perturbation into hepatocytes of living animals and enable identification of perturbations by molecular barcoding. We generate genetic mosaicism by modifying a validated set of 8 cancer-associated genes (e.g. Ctnnb1, Trp53, Myc). Since HTVI achieves multiple genomic integrations per cell, up to 256 different genotypes (all possible combinations of 8 alterations) could theoretically be created within a single mouse liver.

We sequentially process livers over 12 weeks post-HTVI and prepare FFPE-samples followed by consecutive sectioning. We finally nominate select samples for spatial omics readouts, including multiplex IHC/IF and spatial transcriptomics. We anticipate this proof-of-concept experiments to demonstrate that spatial omics can be used to simultaneously identify multiplexed molecular barcodes (i.e. associated combinatorial genetic perturbations) and demarcate cell types of interest (e.g. tumor cells, immune cells) within a native spatial context.
Our approach could allow us to systematically map the microenvironment surrounding genetically heterogeneous liver tumors, thereby substantially advancing the analysis of genotype-to-phenotype relations in a relevant in vivo disease model.

P4.19 LZTR1 acts as a potent tumor suppressor gene in liver cancer by safeguarding aberrant MAPK activity via posttranslational control of RAS GTPases

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DOI 10.1055/s-0042-1760013
Hepatocellular carcinoma (HCC) ranks among the cancers with the highest rate of mortality, yet treating this carcinoma remains challenging due to late diagnosis and poor patient stratification, thereby preventing the utilization of targeted therapeutical approaches. Using human genome sequencing data, we identified frequent deleterious alterations of Leucine-zipper-like transcriptional regulator 1 (LZTR1), which plays a crucial role in regulation of RAS-like GTPases (e.g. RIT1) and downstream pathways, such as the mitogen activated protein kinase (MAPK) pathway. Using murine in vivo as well as human in vitro models, we reveal that loss of function of LZTR1 promotes tumorigenesis in vivo as well as cell growth in vitro, an effect accompanied by elevated RIT1 expression and subsequent MAPK pathway activation. Moreover, truncated forms of LZTR1 lacking domains crucial for its interaction with RAS molecules phenocopied the effect of LZTR1 loss, further suggesting that this interaction is crucial for tumor suppression. Finally, expression of mutant RIT1 proteins rendering RIT1 non-degradable by LZTR1 in murine livers resulted in liver tumorigenesis comparable to LZTR1 loss. Thus, our findings suggest that LZTR1 safeguards MAPK signaling by controlling RAS GTPases in the liver and could therefore potentially be utilized to stratify HCC patients for usage of small molecule inhibitors targeting MAPKs, which are currently only employed in other carcinomas.

P4.20 Repurposing passenger amplifications for specific therapeutic targeting of liver cancer

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Current cancer therapies focus on targeting driver alterations responsible for tumorigenesis. However, these alterations are often not actionable or are only present in a subset of patients. We hypothesized that passenger events in amplified regions could be therapeutically exploited by providing actionable molecules on the cell surface. Using publicly available multi-omics data, we identified the cell-surface protein-coding gene MPZL1 (Myelin protein zero-like 1, located in Chromosome 1q), which is amplified in 75 % of hepatocellular carcinomas (HCCs), accompanied high mRNA expression in tumors compared to normal livers. We further validated MPZL1 protein expression in a wide range of human cancer entities (n = 2038) and normal tissues (n = 163) by immunohistochemistry, and found that a high percentage of tumors present scores 2 or 3 (e.g. 48 % of HCCs), whereas healthy tissues are mostly negative/faintly positive (scores 0 or 1). Next, we generated a monoclonal antibody directed to the extracellular domain of MPZL1, which was then used to generate a CAR (chimeric antigen receptor) construct targeting MPZL1. Corresponding CAR-T cells potently killed various MPZL1-high human cancer cells in vitro, whereas they failed to kill respective isogenic cells with MPZL1 knockout. Moreover, anti-MPZL1 CAR-T cells underwent antigen-dependent proliferation and showed increased cytokines production (IFNγ, TNFα, IL-2, GZMB), further confirming their specificity. In summary, our findings reveal MPZL1 as a new target for the treatment of 1q-amplified cancers and implement a novel immunotherapeutic strategy based on anti-MPZL1 CAR-T cells. Furthermore, our work suggests an innovative approach in drug development by targeting passenger events within large chromosomal amplifications.

P4.21 IDH1 targeting as a potential treatment for intrahepatic cholangiocarcinoma (iCCA)

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Gain-of-function mutations in IDH1 render it with a neomorphic activity to produce an oncometabolite, 2-hydroxyglutarate (2-HG). Little is known about the relevance of this phenomenon in intrahepatic cholangiocarcinoma (iCCA), even though this gene is among the most frequently mutated genes in this tumor type. Furthermore, mutated IDH1 could serve as an important potential target for exploring novel therapeutic options for iCCA.
To elucidate the role of IDH1 in the development of iCCA and determine the functional consequences of 2-HG production, we employed a mouse model which enables introduction of genetic elements directly into the liver. Our results revealed that IDH1 mutations combined with other iCCA-driving oncogenic events shorten survival span of tumor-bearing mice. Moreover, 2-HG accumulation in tumor tissue leads not only to upregulation of methylation and induction of tumor differentiation, but also to altered stromal cell infiltration (e.g. fibroblasts, lymphatics). Further, to identify key players contributing to 2-HG-driven phenotype, we apply mass spectrometry analyses of extracellular matrix from liver cancer tissue. Additionally, to target IDH1 mutant cholangiocarcinoma cells, we screened for peptides with an immunogenic capacity and identified a novel peptide, which is suitable for mutation-specific vaccination. Further experiments are now investigating the therapeutic potential of the novel peptide for rescuing IDH1-related iCCA.
In summary, our results reveal a crucial role of IDH1 in shaping the tumor microenvironment and cell differentiation in iCCA and provide novel insights into immunotherapeutic options for targeting IDH1 as a tumor-specific neoantigen.

P4.22 Combination of panobinostat and bleomycin induces apoptosis in Hepatocellular Carcinoma (HCC) through downregulation of Bcl-XL and Mcl-1

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Background Hepatocellular carcinoma (HCC) is still a challenge in clinical practice, since HCC cells in advanced stages develop an apoptotic resistant phenotype being associated with development and progression of HCC. We evaluated the effect of combining histone deacetylase inhibitors and the chemotherapeutic agent bleomycin on cell death induction in HCC cells. Method: HepG2 cells were incubated with serum concentrations of histone deacetylase inhibitor panobinostat (0.1-5µM) and bleomycin (2-10µM). Cell death was detected after 12 and 24h by flow cytometry using DAPI/Annexin V-APC staining.

The caspase inhibitor Z-VAD-FMK was added to determine the mode of cell death. Caspase cleavage as well as levels of pro-and anti-apoptotic members of the Bcl-2 family were analyzed by Western blot.

Results 24h treatment with 1µM panobinostat induced an increase in cell death of up to 25 % compared to controls. 10µM bleomycin resulted in cell death induction of 35 % of the HCC cells. After incubation with a combination of panobinostat and bleomycin cell death was increased by up to 60 %, indicating a synergistic effect. Cell death could effectively be blocked by zVAD-FMK, suggesting apoptosis as the underlying mechanism. Panobinostat and bleomycin alone as well as the combination of both resulted in a cleavage of caspases-3,-8, and -9, a downregulation of the anti-apoptotic proteins Bcl-XL and Mcl-1 as well as a cleavage of PARP, confirming induction of apoptosis.

Conclusion Panobinostat and bleomycin target different cellular pathways leading to synergistic effects and pronounced induction of apoptosis in HCC, playing an important role for future therapies.

P4.23 Exon-specific isoform expression reporter systems (EXSISERS) – A novel reporter to monitor differential p53 protein-isoform expression in response to tumor-specific treatments

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DOI 10.1055/s-0042-1760017

Introduction Tumor suppressor p53 recognizes cellular stress and regulates cell proliferation and induction of cell death. Specific cell fates are highly dependent on the interplay of distinct p53 isoforms. Hereby, the expressive p53 isoform-ratio can determine the patient's response to a certain systemic therapy. However, differentiating p53 isoform expression in (tumor) cells is challenging. Here, we introduce a novel system using luminescence-coupled split-inteins which are spliced out during translation of p53 and enable detection of all p53 protein isoforms in real-time.

Methods We stably integrated three exon-specific isoform expression reporter systems (EXSISERS) into exon 2, exon 4, and exon 7 of TP53 to differentiate three major protein isoform groups (full length (FL)p53, Δ40p53, Δ133p53) in modified HCT116 colorectal cancer (CRC) cells. Knock in of the corresponding constructs via CRISPR/Cas9 was validated by genotyping.

Results For different CRC-specific drug treatments, we distinguished the cellular amount of FLp53, Δ40p53 and Δ133p53 protein isoforms. 5-FU and Oxaliplatin, which evoke incorrect DNA and RNA synthesis, induced cell cycle arrest and apoptosis by upregulating FLp53 and Δ40p53. Additional screening of over 5'000 active drug compounds regarding differential p53 isoform expression resulted in a selection of highly efficient drugs for the treatment of CRC. We are currently adapting this system to HepG2 hepatocellular carcinoma cells.

Conclusion Implementing EXSISERS in any type of cancer cell line, including hepatocellular carcinoma, and several primary tissues provides novel screening opportunities for p53-dependent drugs and the development of optimized and personalized cancer treatments.

P4.24 A novel microRNA-MAPK14-ATF2 axis contributes to hepatocellular carcinoma progression and sorafenib-resistance

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Introduction Hepatocellular carcinoma (HCC) is a highly malignant and heterogeneous cancer type. Multi-kinase inhibitor sorafenib was the first molecular therapeutic to be approved for advanced HCC. The precise role of stress-induced mitogen-activated kinase 14 (MAPK14) in therapy-resistance is unknown. We aimed at exploring the expression, function, and regulation of MAPK14 in HCC progression and sorafenib-resistance.

Methods Primary human hepatocytes, human HCC cell lines (PLC, Hep3B, HepG2, Huh-7), and sorafenib-resistant Hep3B cells were analyzed. HCC patient tissues and non-cancerous liver were used for mRNA quantification in vivo. MicroRNA-622-mimic and si-RNA-pools targeting MAPK14 and activating transcription factor 2 (ATF2) were used for analysis of regulation and function. Correlation analyses were performed in silico.

Results MAPK14 was strongly upregulated in HCC cells and patient tissues. Tumorsuppressive microRNA-622, which is frequently downregulated in HCC, was identified as post-transcriptional regulator of MAPK14 mRNA. MAPK14 itself can induce activation of ATF2, which was independently found to be a miR-622 target in HCC. MAPK14 and ATF2 levels were significantly downregulated after re-expressing tumorsuppressive miR-622. Luciferase reporter assays confirmed functional miR-622 binding sites in MAPK14 and ATF2 3'UTR sequences. Both MAPK14 and ATF2 were upregulated in sorafenib-resistant compared to non-resistant cells. In silico analyses showed positive correlation of MAPK14 and ATF2 with EMT marker expression in HCC patients.

Conclusions We found a novel miR-622-MAPK14-ATF2 axis in HCC. Both MAPK14 and ATF2 levels are upregulated in HCC and promote sorafenib-resistance. Tumorsuppressive miR-622 was found to target both MAPK14 and ATF2, revealing a novel signaling crosstalk with potential therapeutic relevance.

P4.25 Einsatz der molekularpathologischen Diagnostik beim cholangiozellulären Karzinom (CCA) – Analyse über 2 Jahre (07/2020-10/2022) eines universitären Zentrums.

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Hintergrund Das cholangiozelluläre Karzinom (CCA) gehört zu den seltenen Erkrankungen. Auf Grund limitierter Behandlungsoptionen in späteren Therapielinien wird eine molekularpathologische Diagnostik zur Identifikation molekularer Targets für eine zielgerichtete Therapie empfohlen.

Ergebnisse 36 Analysen im Rahmen der prädiktiven molekularen Diagnostik bei Patienten mit CCA wurden ausgewertet. Mit 69,4 % (n = 25) war der häufigste Subtyp das intrahepatische CCA, gefolgt vom extrahepatischen CCA mit 19,4 % (n = 7) und dem Gallenblasenkarzinom mit 11,1 % (n = 4). In der molekularpathologischen Diagnostik zeigten 72 % der Patienten (n = 26) eine therapeutisch adressierbare molekulare Alteration (FGFR2-Fusion 5,56 % (n = 2), FGFR2-Mutation 2,78 % (n = 1), IDH1-Mutation 13,89 % (n = 5), HER2-Überexpression 5,56 % (n = 2), Mikrosatelliteninstabilität (MSI-high) 2,78 % (n = 1), KRAS/NRAS-Mutation 27,78 % (n = 10)). Weitere detektierte Targets waren BRAF-K601E-Mutationen (n = 2), MET-Fusion (n = 1), EGFR-Amplifikation (n = 1), CHEK2-Mutation (n = 2), BRCA2-Mutation (n = 1), PIK3CA-Mutation (n = 3), TSC1-Mutation (n = 1), POLD1-Mutation (n = 1), ERBB2-Mutation(n = 1). Die hier aufgeführten Mutationen wurden im Rahmen des molekularen Tumorboards als therapeutisch adressierbar eingestuft und mit einer entsprechenden Therapieempfehlung versehen. 8 Patienten konnte eine zielgerichtete Therapie angeboten werden (2x Ivosidenib bei IDH1-Mutation, 1x Pemigatinib bei FGFR2-Fusion, 1x Olaparib bei BRCA-Mutation, 1x Pembrolizumab/Lenvatinib bei MSI-high-CCA, 1x Erlotinib/Bevacizumab bei EGFR-Amplifikation, 1x Trastuzumab/Pertuzumab bei Her2-Überexpression, 1x Binimetinib/Capecitabin bei KRAS-Mutation).

Schlussfolgerung Die zielgerichtete Tumortherapie stellt bei Patienten mit CCA eine wichtige Therapieoption dar. Durch Einführung molekularer Diagnostik konnte für Patienten mit entsprechenden molekularen Alterationen eine

signifikante Verbesserung der Tumorkontrolle sowie ein Erhalt der Lebensqualität erreicht werden. Die frühzeitige molekularpathologische Diagnostik hat sich in unserem Zentrum etabliert. In unserem Patientenkollektiv zeigte sich insgesamt eine hohe Frequenz therapeutisch adressierbarer Targets.

P4.26 Atezolizumab plus bevacizumab beyond first-line in hepatocellular carcinoma

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Introduction Atezolizumab plus bevacizumab (atezo/bev) is the standard of care in first-line systemic therapy for advanced stage hepatocellular carcinoma (aHCC). Data on efficacy and safety of atezo/bev in patients with aHCC who received previous systemic therapy are not available.

Methods Patients with aHCC who received atezo/bev after at least one systemic treatment between December 2018 and March 2022 were retrospectively identified across 13 centers in Germany and Austria. Patient characteristics, tumor response rates, pro-gression-free survival (PFS), overall survival (OS) and adverse events (AE) were analyzed.

Results Overall, 50 patients were identified, 41 (82 %) were male. The median age at the start of atezolizumab plus bevacizumab was 65 years, 41 (82 %) patients had liver cirrhosis, 30 (73 %) Child A, 9 (22 %) B and 2 (5 %) C. Overall, 34 patients (68 %) received atezolizumab plus bevacizumab in second-line and 16 (32 %) in later lines. Best radiological tumor responses were complete remission (2 %), partial remission (30 %), stable disease (36 %) and progressive disease (18 %), resulting in an objective response rate of 32 % and a disease control rate of 68 %. Median OS was 16.0 months (95 % CI 5.6-26.4 months), and mPFS was 7.1 months (95 % CI 4.4-9.8 months). AE grade 3-4 were observed in 7 (14 %) and led to death in 3 patients (6 %). There were 5 (10 %) bleeding events of grade ≥ 3, one of them (2 %) with fatal outcome.

Conclusion Atezo/bev is effective beyond first-line in aHCC. The safety profile was consistent with previous reports.

P4.27 The combination of EpCAM-positive circulating tumor cells and serum AFP levels predicts early recurrence after resection of hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) has high recurrence rates of more than 50 % after tumor resection. As reported earlier, EpCAM-positive circulating tumor cells (CTC) have a high predictive value for presence of micro-metastases and early HCC recurrence after curative resection, however, sensitivity remains low. The serum biomarker alpha-fetoprotein (AFP) is a well-known diagnostic and prognostic marker for HCC. The objective of this study was to evaluate a composite test of CTC and AFP to identify patients with high risk of early recurrence within two years after liver resection.

We prospectively enrolled 58 patients undergoing curative intended resection for HCC at a tertiary referral center. Blood specimens were obtained prior to resection and analyzed for EpCAM-positive CTC and serum AFP levels. Primary endpoint was early recurrence-free survival (RFS). 49 patients with completed follow-up were analyzed.

A positive test was defined as detection of any CTC or AFP levels > 400 ng/ml. Eight patients tested positive for CTC, seven for AFP and two for both markers. CTC and AFP positive patients had a high probability of an early recurrence with a positive predictive value of 86 % each. However, sensitivity was limited with 21 % and 18 %, respectively. The combination of both markers increased the sensitivity to 33 %, while retaining a positive predictive value of 84 %. A positive composite test was significantly associated with shorter RFS (5 vs. 13 months, p = 0.009).

The combination of EpCAM-positive CTC and AFP serum levels improved the sensitivity for the prediction of early recurrence, while maintaining a high positive predictive value.

P4.28 Spatiotemporal analysis of tumour-infiltrating immune cells in biliary carcinogenesis

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Intraductal papillary neoplasms (IPN) and biliary epithelial neoplasia (BilIN) are distinct precursor lesions that may progress to biliary tract carcinoma (BTC). A comprehensive characterization of the inflammatory microenvironment of IPN and BilIN precursor lesions was the main aim of our study. Immunohistochemistochemical staining with anti-CD3, -CD4, -CD8, -CD20, -CD68, -CD163, -CD56, and -MUM1 antibodies was used to investigate tumor-infiltrating immune cell populations in tissue samples from patients in whom coexisting precursor lesions and invasive BTC were identified. Using a triplet sample set of nonneoplastic epithelium, precursor lesions, and invasive BTC, we extensively analyzed the spatiotemporal evolution of the immune microenvironment during IPN- and BilIN-related carcinogenesis. We found that stromal CD3 + (P = 0.002), CD4 + (P = 0.007) and CD8 + (P < 0.001) T cells were significantly reduced in IPN compared to nonneoplastic epithelium. Similarly, fewer CD20 + B cells (P = 0.008), MUM1 + plasma cells (P = 0.012) and CD163 + M2-like macrophages (P = 0.008) were observed in IPN compared to nonneoplastic epithelium. Stromal CD68 + (P = 0.001) and CD163 + (P < 0.001) macrophages significantly increased during the transition from IPN to invasive BTC. Intraepithelial CD4 + and CD8 + T cells conversely decreased from nonneoplastic epi-

thelium to IPN and were similarly distributed in invasive BTC. BilIN-associated biliary carcinogenesis was characterized by a significant reduction of intraepithelial CD8 + T-lymphocyte infiltration at the transition from non-tumorous biliary epithelium to BilIN(P = 0.008) and a further decrease from BilIN to BTC (P = 0.004). We conclude that IPN and BilIN undergo distinct immune-cell changes throughout biliary carcinogenesis

P4.29 Multidisciplinary approach for biliary tract cancer after progression to first-line therapy with gemcitabine and cisplatin: post-progression survival and safety outcomes

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Background Patients with unresectable biliary tree cancer (uBTC) progressing despite first-line treatment with gemcitabine plus cisplatin (GC) have limited systemic options with modest survival benefit. Data on efficacy and safety of a personalized treatment based on a multidisciplinary discussion for patients with progressing uBTC are lacking.

Methods In this retrospective single-center study, patients with progressing uBTC who received either best supportive care or a personalized treatment based on a multidisciplinary discussion (i.e. local-ablative therapy, FOLFIRI or both) between 2011 and 2021 were included.

Results Ninety-seven patients with progressing uBTC were identified. Patients received best supportive care (N = 50, 52 %), local-ablative therapy (N = 14, 14 %), FOLFIRI (N = 19, 20 %) or both (N = 14, 14 %), respectively. Post-progression survival of patients receiving local-ablative therapy (8.8 months; 95 % CI: 2.60-15.08), FOLFIRI (6 months; 95 % CI: 3.30-8.72) or both (15.1 months; 95 % CI: 3.66-26.50) was significantly longer compared to patients receiving BSC (0.36 months; 95 % CI: 0.00-1.24, p < 0.001). The most common (> 10 %) grade 3-5 adverse events were anemia (25 %) and thrombocytopenia (11 %).

Conclusion Multidisciplinary discussion is crucial for identifying patients with progressing uBTC that may most benefit from local-ablative therapy, FOLFIRI or both. The safety profile was consistent with previous reports.

P4.30 The predictive value of serum Dickkopf-related protein 1 in evaluation tumor response to transarterial radioembolization in patients with hepatocellular carcinoma

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Background In the treatment of hepatocellular carcinoma (HCC) response to transarterial radioembolization (TARE) is variable and poorly predictable. Since we know from previous studies that Dickkopf-related protein 1 (DKK-1) can be a good prognostic biomarker for the response of several HCC therapies, we aimed in this study to correlate circulating DKK-1 with patient and tumor characteristics and response to subsequent TARE in a European population of HCC patients. **Methods** Samples from HCC patients treated with TARE (n = 43) at Leipzig University Hospital between 2010 and 2020 were retrospectively analyzed. Levels of DKK-1 were measured in serum samples collected before and after TARE. Tumor response was assessed according to mRECIST criteria at week 12 after TARE. DKK-1 levels at baseline and at week 12 after TARE were assessed for their association with response (deltaDKK-1).

Results Before TARE, mean DKK-1 serum levels were 2594 ± 2815 (811-19062) pg/mL and after TARE, mean DKK-1 serum levels were 2043 ± 1738 (501-10514) pg/mL (p < 0.001). DKK-1 levels before TARE were significantly lower in patients showing response at week 12 as compared to patients without response at week 12. Changes in DKK-1 serum levels were significant different in the response group compared to the non-response group (p = 0.047). ROC curves revealed that levels deltaDKK-1 had a stronger association with response to TARE (AUC = 0.760, p = 0.02) than DKK-1 before (AUC = 0.723, p = 0.097) and after TARE alone (AUC = 0.548, p = 0.78).

Conclusion Our results demonstrated that serum DKK-1 levels before TARE and changes after TARE are biomarkers for reponse to TARE in patients with HCC.

P4.31 Poor response to sorafenib in HCC patients induces distinct immune-related signatures and is mediated by hypoxia-related 14-3-3 scaffolding proteins

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Introduction Poor response to the drug treatment or relapse quickly after initial remission are events frequently observed in an advanced stage of hepatocellular carcinoma (HCC).

Aims Our goal was to dissect molecular drivers of poor response to sorafenib treatment and to identify novel prognostic markers associated with distinct tumor immune landscapes.

Methods From a cohort of 91 patients treated with sorafenib, we identified 17 HCC patients with particularly good or bad response. Integrative RNA sequencing and whole-exome sequencing analyses were performed to identify predictive markers of sorafenib resistance. In vitro validations of defined targets were performed in a model of sorafenib resistance, followed by subsequent functional and mechanistic validation.

Results Patients with worst response (n = 7) were characterized by significantly shorter treatment duration and poor overall survival than good responders (n = 10). Molecular analyses revealed that acquisition of drug resistance observed in poor responder group was associated with upregulation of hypoxia-related targets from 14-3-3 scaffolding protein family. Specific peptide inhibition of these proteins, in combination with sorafenib, displayed synergistic effects and efficiently reduced cell proliferation and viability. Dual inhibition consequently reversed sorafenib resistance in both, normoxic and hypoxic conditions. Furthermore, a shift in immune-cell composition with predominant enrichment of M2-immunosuppressive macrophages in worst responders was observed.

Conclusion Defining actionable targets and their subsequent inhibition might greatly help delineate molecular alterations driving drug resistance. Further, characterization of the immune micro-milieu in different subgroups could be of particular importance to depict treatment resistance and warrants further investigations.

P4.32 Re-programmierung der Nekroptose limitiert Immunreaktionen und verhindert die Entstehung von Leberkrebs

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Der Übergang von einer chronischen Leberschädigung hin zum Krebs wird durch immunologische Reaktionen auf verschiedene Formen des Hepatozytensterbens angetrieben, die durch bestimmte pathogene Reize ausgelöst werden. Während man davon ausgeht, dass die Apoptose eine minimale Auswirkung auf die Entzündung hat, ist es ein allgemeines Dogma, dass die Nekroptose, eine Form der programmierten Nekrose, die von der RIPK3- und MLKL-Aktivierung abhängt, hoch reaktiv ist und die Entzündung durch nicht genau definierte Effektormoleküle auslöst.

Wir zeigen nun, dass zwei Formen der Nekroptose in Leberparenchymzellen (LPC) existieren, die bei chronischen Leberschäden entgegengesetzte immunologische Reaktionen hervorrufen. In einem spezifischen molekularen Kontext löst die LPC-Nekroptose eine massive kompensatorische Proliferation von Hepatozyten und biliären Vorläuferzellen aus, die mit der Aktivierung spezifischer Untergruppen myeloischer Zellen einhergeht, die wir durch Einzelzell-RNA-Seq. charakterisiert haben. Diese „hyper-reaktive“ Form der Nekroptose führt zu Leberkrebs und zystischer Degeneration des Gallengangssystems. Folglich konnten wir eine "hyper-reaktive Nekroptose-Signatur" bei menschlichem Leberkrebs definieren, die mit einer schlechten Prognose assoziiert ist. Interessanterweise existiert ein molekularer Schalter in Hepatozyten, der die Nekroptose in eine „hypo-reaktive“ Form des Zelltods umwandelt, die durch eine unzureichende Immunaktivierung charakterisiert ist und demzufolge die Hepatokarzinogenese verhindert.

Somit bestimmt die Reaktivität der Nekroptose ihr karzinogenes Potenzial, und die "Re-programmierung" der Nekroptose könnte eine neue Strategie der Chemoprävention gegen entzündliche Krebserkrankungen darstellen.

P4.33 The GALAD score is associated with response to loco-regional and systemic therapies for hepatocellular carcinoma

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Background The GALAD score is a biomarker-based scoring system used for detection of hepatocellular carcinomas (HCC). It consists of gender (G), age (A) and levels of AFP-L3 (L), AFP (A), and DCP (D). So far, a possible association of GALAD score and therapy response was not investigated. The study aim is to assess the value of the GALAD model as a response marker to loco-regional treatments and systemic therapies for HCC.

Methods In a retrospective study, 235 patients with HCC treated with loco-regional treatment or systemic drugs were enrolled. Serum samples at baseline were analyzed. Treatment efficacy was evaluated by mRECIST criteria three months after intervention.

Results At baseline, GALAD scores were significantly higher in the refractoriness group compared to response group ($p < 0.001$). Regarding the different therapy options, the GALAD score showed a significant discrimination between refractory and responder in the loco-regional therapy group ($p < 0.001$) as well as in the systemic treatment group ($p = 0.01$). Using ROC analyses the GALAD score for response of loco-regional or systemic treatment showed higher AUC (AUC = 0.704; $p < 0.001$) than the individual biomarkers AFP, AFP-L3, and DCP. Moreover, we analyzed the GALAD score in a subgroup of patients with AFP levels < 130 ng/ml and were able to show that the baseline GALAD is also associated with a response in these patients ($p = 0.008$).

Discussion The GALAD model at baseline is a potent predictor of tumor response to loco-regional and systemic treatment in HCC in patients.

P4.34 Preferential effect of PARP-1 inhibition in KRAS-mutated intrahepatic cholangiocarcinomas is mediated by CHK1/2 kinases

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Activating KRAS mutations are among the most abundant genetic alterations in intrahepatic cholangiocarcinoma (iCCA) and are associated with early recurrence, poor response, and reduced survival. Poly(ADP-ribose)polymerase-1 (PARP-1) is frequently observed to be upregulated in iCCA. Evidence indicate potential therapeutic relevance for PARP-1 inhibition in iCCA that preferentially affects KRAS-mutated cancers, but exact mechanisms remain unknown.

PARP-1 depletion was generated by siRNA and CRISPR/Cas9-mediated knockdown/knockout, followed by molecular and functional analyses. To investigate impact of PARP-1 deficiency in KRAS-driven tumorigenesis, PARP-1 knockout mice were combined with inducible KRAS-driven mouse model using hydrodynamic tail vein injection. Transcriptome analyses were employed to further investigate molecular mechanisms.

Significant upregulation of PARP-1, as well as enrichment of genes related to PARP-1 activation, was observed in iCCA tissue and KRAS-mutated cell lines. Knockout of PARP-1 in KRAS-mutated cells led to reduction in colony and sphere formation. Moreover, KRAS-mutated cell lines showed higher sensitivity to PARP-1 inhibition. In vivo PARP-1 deficiency considerably impaired biliary carcinogenesis and induced shift from dominant iCCA towards HCC phenotype in KRAS-dependent manner. Transcriptome analyses of CRISPR/Cas9 PARP-1 knockout clones and in vivo tumors revealed differential expression of DNA damage response pathways (e.g. CHK1/2) as well as cellular pathways affected by PARP-1, (inflammation, oxidative stress, cell death signaling). The most prominent candidate regulating PARP1 in KRAS cell lines and tumors appeared to be CHK1/2 kinases, further validated by qRT-PCR, western blot, and drug-screening assays.

Together, these findings suggest an unrecognized prognostic and therapeutic role of PARP-1 in iCCA patients with oncogenic KRAS signaling.

P4.35 Sarcopenia indicate poor survival in patients undergoing transarterial chemoembolization (TACE) for hepatic malignancies

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Background Patient selection for transarterial chemoembolization (TACE) has remained challenging. The patients´ body composition has been linked with patient outcome in different cancers, but its role in the context of TACE is only poorly studied. Here, we evaluated the role of the body composition as prognostic parameter in patients undergoing TACE for primary and secondary liver cancer.

Methods Pre-interventional CT-scans were used to assess five parameters of the individual body composition (skeletal muscle index (SMI), median muscular attenuation, bone mineral density as well as the visceral and subcutaneous fat area) in 89 patients undergoing TACE. Results were correlated with tumor response to TACE and outcome of patients.

Results SMI and visceral fat area were significantly higher in male patients and among patients undergoing TACE for HCC compared to patients with liver metastases. Patients with an SMI below the ideal cut-off value of 37.76cm2/m2 had a significantly reduced long-term outcome with a median overall survival of 404 days compared to 1321 days for patients with a high SMI. Moreover, the pre-interventional SMI turned out as an independent prognostic factor in a multivariate Cox-regression model including clinicopathological parameters and laboratory markers of organ dysfunction and systemic inflammation (HR: 0.899, 95 %CI: 0.827-0.979, p = 0.014).

Conclusion The pre-interventional SMI represents an independent prognostic factor for overall survival following TACE for primary and secondary liver cancer. Assessment of the individual body composition using routine CT-scan might help to identify the ideal candidates for TACE in future.

P4.36 Micro-RNA Landscape of Hepatocellular Carcinoma-Derived Extracellular Vesicles

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Background Extracellular vesicles (EVs) produced by cancer cells contain bioactive cargo, including micro-RNAs (miRNAs), which can both provide insights into tumor cell communication but may also serve as a biomarker for liquid biopsy. We therefore asked if HCC tumor cell line EVs could be utilized to identify HCC-related patient miRNA profiles in liquid biopsy.

Methods EVs were extracted from the supernatants of different HCC-cell lines cultured in 2D and 3D models. These culture-derived vesicles were analyzed using Nano tracking analysis (NTA), mass spectrometry, proteomics and miRNA sequencing. MiRNA-sequencing was also performed on blood samples of HCC patients (n = 20) and healthy donors (n = 7). Cell lines were further characterized by mass cytometry, RNA-sequencing, and whole-exome-sequencing.

Results Comparison of miRNA profiles between 2D and 3D cultures identified a conserved miRNA pattern. The analysis of HCC cell line-derived extra vesicular-, patient-, and healthy donor-miRNAs identified an HCC-specific signature consisting of 75 miRNAs. Pathway analysis informed that these miRNAs can regulate multiple cellular checkpoints for HCC-development.

Conclusion We identified an extra-vesicular HCC miRNA signature that is conserved in HCC cell lines and enriched in HCC patient peripheral blood. These findings suggest that HCC EVs are informative for liquid biopsy-based diagnostics. They also propose that extracellular vesicles may function as systemic mediators of HCC tumor cell communication.

P4.37 Analysis of SH2D4A promoter activity reveals positive regulation by inflammatory cytokines via transcription factor KLF4.

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Hepatocellular carcinoma is typically preceded by chronic liver damage and inflammation. Cytokines associated with tumor initiation, invasion, and metastasis are often elevated in the tumor microenvironment. SH2D4A acts as a tumor suppressor in HCC, shows a reduced expression in tumor tissue and can be used to predict survival. In this study we aimed to explore the transcriptional regulation of SH2D4A expression in HCC.

We found that after stimulation with cytokines IFN-γ and TGFβ, SH2D4A expression is significantly increased on mRNA level. Further studying the downstream regulators, the SH2D4A promoter emerged to be regulated by transcription factors FOXA1, YY1 and KLF4. Systemic study of KLF4 binding sites revealed two directly adjacent binding sites with significant impact on SH2D4A promoter activity in HepG2 cells. One binding site exhibited a direct positive effect on SH2D4A promoter activity when stimulated, the other binding site conveyed an increase in promoter activity by disinhibition. A decrease in SH2D4A promoter activity not associated with a specific binding site indicated a cooperative interaction in Hep3B cells. Gene expression data analysis demonstrated several differentially expressed KLF4 target genes associated to SH2D4A expression. Of those genes, Phosphatidylinositol 3-kinase regulatory subunit alpha (PIK3R1) and Dual specificity protein phosphatase Cell Division Cycle 14B (CDC14B) showed a significant positive association with overall survival probability.

We demonstrated an influence of cytokines on the endogenous SH2D4A expression activity and uncovered a regulatory interaction between KLF4 and the SH2D4A promoter. Understanding the regulation SH2D4A is key to find new innovative therapeutic approaches for targeting cancer cells.

P4.38 Strong efficacy and tolerable safety profile for the combination of atezolizumab and bevacizumab in hepatocellular carcinoma after previous systemic therapy

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Background Atezolizumab and bevacizumab (atezo/bev) has become the novel standard as first-line treatment for advanced hepatocellular carcinoma (HCC). However, data is lacking for patients previously treated with tyrosine kinase inhibitors (TKI).

Methods In this prospective single-center observational study, we analysed 16 patients receiving atezo/bev after previous systemic therapy. Primary endpoints were overall survival (OS) and progression free survival (PFS).

Results All 16 patients received prior systemic therapy (94 % TKI, range 1-6 lines). Median age was 68 years, 88 % of patients were male. Ten patients (62 %) were classified as Child-Pugh stage (CPS) A, five (31 %) as CPS B, and one (6 %) as CPS C. 13 (81 %) patients were BCLC stage C, and three (19 %) were BCLC B with progression after locoregional therapy. Median OS and PFS were 6.3 (95 %-CI 3.6-not estimable [NE]) and 4.7 months (95 %-CI 2.2-NE), respectively. Five (31 %) patients had complete or partial response, three (19 %) had stable

disease, and four (25 %) had progressive disease according to RECIST (four not assessable). Noteworthy, patients with CPS A had significantly longer OS (NE vs 2.43, p = 0.023) and PFS (10.6 vs 2.03 p = 0.015) compared to patients with Child B/C.

Overall, seven (44 %) patients experienced any treatment-related adverse event (trAE) (19 % of patients developing hypertension, 25 % proteinuria,13 % bleeding, 19 % fatigue, 19 % immune-related adverse events (irAE)), with only one patient experiencing trAE grade ≥ 3.

Conclusion Our results indicate strong efficacy and acceptable toxicity for atezo/bev in HCC patients with CPS A after prior exposure to systemic TKI therapy.

P4.39 Expression of growth differentiation factor 5 in liver fibrosis and cancer

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The activation of hepatic stellate cells (HSC) is the key event of hepatic fibrosis and activated HSC also play a critical role in the progression of hepatocellular cancer (HCC). Growth differentiation factor 5 (GDF5) belongs to the TGF-β/BMP-superfamily. So far, GDF5 has been mostly studied in the context of cartilage development and repair.

The aim of this study was to assess the expression and function of GDF5 in liver fibrosis and cancer.

Methods and results GDF5 expression-levels increased during in vitro activation of primary human HSC and in different murine models of hepatic fibrosis. Furthermore, there is a significant correlation between the expression of GDF5 and alpha-smooth-muscle actin (a-sma) in human HCC as well as non-tumorous liver tissues. RNAi mediated GDF5-suppression in activated HSC resulted in reduced a-sma and collagen-expression, while treatment with recombinant GDF5 induced a-sma and collagen-expression. In human HCC cell-lines treatment with recombinant GDF5 induced the phosphorylation of Smad1/5/8 and the expression of the transcription factors inhibitor of differentiation 1 (ID1), a known promoters of HCC-progression. In line with this, GDF5 induced the proliferation and colony formation of HCC cells.

Summary and conclusion Our data indicate activated HSC as major cellular source of enhanced GDF5 expression in fibrotic liver disease and HCC and show that GDF5 exhibits pro-fibrogenic as well as pro-tumorigenic effect. Future analyses will reveal the potential of this soluble growth-factor as therapeutic target or prognostic marker for fibrosis and HCC-progression in patients with chronic liver disease.

P4.40 Characterization of biological activities of Oncostatin M variants

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Background Oncostatin M (OSM) plays an important role in liver development, liver metabolism and the acute phase response. A special feature of OSM among all IL-6 family members is the presence of a pro-domain in the C-terminal region, which indicates that the cytokine might be processed to obtain its full biological activity. This, however, remains unaddressed to date.

Methods Human hepatoma cells (HepG2), human adenocarcinoma cell (A549) and human melanoma cells (A375) were stimulated with four different OSM variants and in four different concentrations. Western blot analyses determined the activation of signaling pathways. Real-time PCR was utilized to quantify expression of the OSM receptor components and MTT proliferation assays to investigate the growth inhibitory activity.

Results Four recombinant human OSM proteins that differ in length and glycosylation status were tested for their signaling capabilities on the three different human tumor cell lines. All OSM isoforms were able to activate the OSMR complex and to stimulate the JAK/STAT, MAPK, PLC-gamma and/or PI3K/AKT pathway. Despite substantial differences in protein size rather subtle differences, both quantitatively and qualitatively, were detected for the four variants. Quantitative differences emerged between glycosylated and unglycosylated OSM. Proliferation assays revealed a stronger inhibitory effect of the short version of OSM (196 aa) compared to the long OSM variant (227 aa), irrespective of its glycosylation status.

Conclusion Presence or absence of the pro-domain in OSM has only minor impact on its signaling capacities, however, it appears to influence its growth inhibitory activity by mechanisms that need to be determined.

P4.41 IL-1 receptor type 1 (IL-1R1) signaling in hepatocytes is involved in liver cancer development from non-alcoholic fatty liver disease (NAFLD)

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Aim The current study explored the therapeutic value of IL-1R1 blockade in hepatocytes in two models of NAFLD-associated hepatocarcinogenesis.

Methods 1) DEN was used starting at 2 weeks of age in male hepatocyte-specific IL-1R1-deficient (KO) mice and WT littermates. From 6 weeks of age mice were fed a high-fat, high-carbohydrate diet (HFD) or a corresponding control diet (CD) up until 24 weeks of age. 2) Low-dose weekly CCl4 in combination with a high-fat, high-fructose, high-cholesterol western diet (WD) or CD was given to 8-week-old, male KO and WT mice for 12 weeks.

Results All DEN-treated mice fed a HFD developed an obese phenotype and macrovesicular hepatic steatosis with mildly elevated ALT, however IL-1R1 deficiency improved insulin resistance and prevented injurious hepatic JNK activation from DEN + HFD. Additionally, the number of macroscopically visible tumor nodules in KO mice was reduced by 43 % compared to WT littermates with a significant reduction of tumor nodules > 1mm. This translated in a significant reduction of tumor load in the KO. Only WT mice showed an infiltration of CD8 + T cells and MDSCs from DEN + HFD. In the CCl4 model, the addition of a WD did not produce the same extent of metabolic derangement and liver histology and ALT levels were comparable between both genotypes. As seen with the DEN + HFD model, WT mice developed a higher number of dysplastic nodules > 1mm compared to the KO (62.5 % vs. 28.6 %).

Conclusion The inhibition of IL-1R1 signaling specifically in hepatocytes could be an adjunctive concept to current immunomodulation in NAFLD-HCC treatment.

P4.42 Smyd2 As A New Therapeutic Target For Hepatocellular Carcinoma

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Introduction The development of new treatments for advanced hepatocellular carcinoma (HCC) remains urgent due to the limited impact of available therapies patient survival. The methyltransferase SMYD2 acts as an oncogene in numerous types of cancers, but its involvement in HCC remains elusive. Thus, we aimed to assess the therapeutic potential of SMYD2 pharmacological inhibition in HCC.

Methods Public HCC transcriptomic datasets were used to explore SMYD2 expression levels and its correlation with relevant pathways. The effect of treatment with SMYD2 inhibitors (AZ505 and LLY507) on cell viability, apoptosis

and cell cycle progression of HCC cells was evaluated by MTT assay and flow cytometry. RNA-Seq analysis of HuH7 cells treated with LLY507 revealed the underlying mechanism upon SMYD2 inhibition. In vivo therapeutic potential of SMYD2 inhibitors was evaluated on an orthotopic HCC model in C3H/HeN mice.

Results SMYD2 is significantly overexpressed in HCC tissues and negatively correlates with immune-related genes and apoptotic processes that are downregulated in HCC. SMYD2 inhibition by LLY507 induces cell cycle arrest and apoptosis on HCC cells. RNA-seq of LLY507-treated HCC cells unveiled the downregulation of aggressive and cell cycle-related genesNotably, LL507 and AZ505 achieved a strong inhibition of tumor growth in vivo.

Conclusions Bioinformatic analysis of public human HCC datasets indicates that SMYD2 could be a crucial epigenetic regulator involved in HCC progression. Targeted inhibition of SMYD2 exerts a potent antitumoral effect both in vitro and in vivo and reverts oncogenic transcriptional programs, suggesting that SMYD2 merits further investigation as a therapeutic target for HCC.

P4.43 Changing treatment landscape associated with improved survival in advanced hepatocellular carcinoma: a nationwide, population-based study

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DOI 10.1055/s-0042-1760037

Background The treatment of hepatocellular carcinoma (HCC) is undergoing a historic transformation with the availability of several new systemic therapies. Sorafenib represented the only effective treatment option available for a long time. In the last few years, however, a number of new systemic therapies have been proven effective in large clinical trials and gained approval. The impact of this changing landscape has not yet been studied in a German cohort in a nationwide, real-world setting.

Methods This observational, retrospective study is based on a claims data base representative for the German population. To investigate the effect of HCC systemic therapies in this dataset, we selected all HCC patients between 2015 and 2020 (ICD-10-GM C22.0). The study group was divided into two groups: Group A (2015 – 07/2018) consisted of patients receiving systemic therapy prior approval of Lenvatinib and, Group B (08/2018 – 2020) receiving new therapeutic options such as Lenvatinib. OS was estimated with Kaplan-Meier analysis, adjustment for demographics and comorbidities was performed. Annual treatment costs were calculated.

Results In the dataset, a total of 460 HCC patients received systemic therapy in 2015-2020. Based on the date of first drug administration, 255 patients received their treatment prior to approval of Lenvatinib (group A), and 205 afterwards (group B). Patient characteristics were distributed equally. Median overall survival was markedly increased in group B. Prolonged patient survival was associated with higher treatment costs.

Conclusions The introduction of multiple new treatment options resulted in substantial survival improvements of patients with aHCC in Germany.

P4.44 Tumor-directed drug delivery is improved upon intermittent fasting

Authors Becker Svea, Trautwein Christian, Biancacci Ilaria, Möckel Diana, Wang Qingbi, May Jan-Niklas, Su Huan, Candels Candels Lena S., Berres Marie-Luise, Kießling Fabian, Hatting Maximilian, Lammers Twan

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DOI 10.1055/s-0042-1760038

Background Tumor cells rely on the Warburg effect to maintain their high proliferative activity, making them dependent on external glucose supply for cell metabolism and proliferation. Therefore, fasting may have health benefits to combat tumor growth. It can further reduce toxicity and improve the efficacy of chemotherapy. This makes it a favorable approach for cancers with limited systemic and local ablative therapies, such as hepatocellular carcinoma (HCC).

Methods The effects of intermittent (IF) and short-term (STF) fasting were investigated on tumor growth, development of the tumor microenvironment (TME) and tumor-targeted drug delivery in two different murine hepatocellular carcinoma models - subcutaneous and intrahepatic model. Animals were subsequently subjected to IF for 24 days or to STF for 1 day. Cy7-labeled liposomes were applied i.v. and their biodistribution and tumor accumulation was examined via combined micro-CT and fluorescence tomography (µCT-FLT). These effects were validated using immunohistochemistry staining and fluorescence microscopy. Hep-55.1C HCC cells were used to study Cy7-labeled liposomes uptake and to study its molecular mechanism of uptake.

Results Micro-CT and fluorescence tomography (µCT-FLT) demonstrated that IF has a significant effect to slow down tumor growth compared to STF and animals feed ad libitum. Immunohistochemistry staining and fluorescence microscopy revealed significant changes in tumor microenvironment as evidenced by decreased extracellular matrix e.g. collagen production and increased angiogenesis. These changes contributed to increased liposome uptake in IF treated animals using both in vivo models.

In vitro, Hep-55.1C were fasted, stimulated and liposome uptake was analyzed via flow cytometry and immunofluorescence microscopy. Together, the in vitro data demonstrate that the fasting equivalent forskolin causes a significant increase in liposome uptake, which could be reversed specifically by nystatin inhibition. These results identified that the increase in liposome uptake in HCC cells is mediated by caveolar endocytosis.

Conclusion Intermittent fasting improves tumor-directed targeting by increasing angiogenesis and modifying intracellular mechanisms using a caveolar endocytosis-dependent mechanism, making it a promising approach for HCC targeted therapy.

Poster Visit Session V Viral Hepatitis and Immunology
28/01/2023, 11.00 am – 11.45 am

P5.01 Regulation of immune response and hepatic fibrosis by the cytotoxic effector molecules granzyme B and TRAIL in sclerosing cholangitis in mice

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DOI 10.1055/s-0042-1760039

Background Primary sclerosing cholangitis (PSC) is a difficult to treat chronic inflammatory cholestatic liver disease. We observed an increased interferon (IFN)γ response in PSC patients and in a mouse model of sclerosing cholangitis. IFNγ induced expression of the cytotoxic effector molecules granzyme B (GzmB) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in hepatic

NK and CD8 + T cells and mediated liver fibrosis in Mdr2-/- mice. Here, we investigated the significance of GzmB and TRAIL for PSC progression in Mdr2-/- mice.

Methods NK and CD8 + T cells were depleted by antibodies in Mdr2-/- mice. Mdr2-/- x GzmB-/- and Mdr2-/- x Tnfs10-/- mice were generated and analysed at 12 weeks. NK and T cell activation, cytotoxicity and survival were determined by flow cytometry. Liver injury was determined by transaminases and fibrosis markers. Stellate cells and cholangiocytes were analysed by immunohistochemistry.

Results Lack of GzmB as well as depletion of NK and CD8 + T cells provided an anti-fibrotic effect in Mdr2-/- mice. In contrast, liver inflammation in Mdr2-/- mice was enhanced in the absence of TRAIL, which was associated with increased frequencies of mature DCs and activated T cells and elevated cytotoxicity and survival of hepatic CD8 + and CD4 + T cells. Moreover, numbers of stellate cells and cholangiocytes were enhanced and hepatic fibrosis was aggravated in Mdr2-/- x Tnfs10-/- mice compared to Mdr2-/- mice.

Conclusion While GzmB mediates liver cell death and fibrosis in sclerosing cholangitis, TRAIL is induced to dampen T cell responses, stellate cell and cholangiocyte proliferation and subsequent fibrosis.

P5.02 Janus kinase inhibitors modulate hepatitis E virus infection

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DOI 10.1055/s-0042-1760040

Hepatitis E usually is a self-limiting disease, but especially immunocompromised individuals are at risk to develop a chronic and severe course of infection. Janus kinase inhibitors (JAK-I) are a novel drug class for the treatment of autoimmune inflammatory rheumatic disease (AIRD). As JAKs play a key role in innate immunity, viral infections and reactivations are frequently reported during JAK-I treatment in AIRD patient. With the aim to assess the risk of HEV infection during JAK-I therapy, we monitored the presence of HEV RNA, HEV-Antigen and anti-HEV IgG/IgM in RA patients receiving JAK-Is. Furthermore, we identified a AIRD patient under JAK-I therapy with hepatitis E and increased liver enzymes. Transcriptomic analysis of primary human hepatocytes (PHHs) revealed an induction of antiviral programs during HEV infection. This induction was perturbed in the presence of a JAK-I, concomitant with strong elevation of HEV RNA levels. In line, infection experiments displayed an up to 50-fold increase of progeny virus production during JAK-I treatment indicating that JAK signaling is critical to control HEV infection. As our data raise potential concern, screening for HEV seroprevalence and HEV RNA should be considered prior starting JAK-I treatment and in case of elevated liver enzymes during JAK-I therapy.

P5.03 Antiviral cure and liver cirrhosis change the serum cholesteryl ester profile in patients with chronic hepatitis C

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DOI 10.1055/s-0042-1760041

Background Infection with hepatitis C virus (HCV) lowers serum cholesterol levels, which recover during therapy with direct-acting antivirals (DAAs). Serum cholesterol is also reduced in patients with liver cirrhosis. Studies investigating serum cholesterol in patients with chronic liver diseases do mostly not account for the individual cholesteryl ester (CE) species, which have different properties according to acyl chain length and desaturation.

Methods Free cholesterol (FC) and 15 CE species were quantified by flow injection analysis high-resolution Fourier Transform mass spectrometry in serum of 178 patients with chronic HCV before and during therapy with DAAs.

Results Serum CE levels were low in patients with liver cirrhosis compared to patients without cirrhosis. The percentages of CE16:0 and 16:1 were higher and that of CE20:4 and 20:5 were reduced. FC levels were unchanged, and thus the CE/FC ratio was low in cirrhosis. Four CE species were reduced in genotype 3 compared to genotype 1 infected patients. During DAA therapy, nine of the 15 measured CE species, and the CE/FC ratio, increased. Relative to total CE levels CE16:0 declined and CE18:3 was higher at therapy end. At this time % CE14:0, 16:0 and 16:1 were higher and % CE20:4 and 22:6 were lower in the cirrhosis than the non-cirrhosis patients. Viral genotype associated changes of CEs disappeared at therapy end.

Conclusions Serum CE composition differs between patients with and without liver cirrhosis, and also changes by efficient elimination of HCV. Overall, HCV infection and cirrhosis are associated with a less favourable CE profile.

P5.04 Efficacy and Safety of Bulevirtide Monotherapy Given at 2 mg or 10 mg Dose Level Once Daily for Treatment of Chronic Hepatitis Delta: Week 48 Primary Endpoint Results from a Phase 3 Randomized, Multicenter, Parallel Design Study

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Bulevirtide (BLV) was conditionally approved in the EU for the treatment of chronic hepatitis D virus (HDV) infection (CHD). A 24-week (W) analysis of a Phase 3 study (MYR301; NCT03852719) of BLV 2 or 10 mg QD demonstrated significantly greater combined virologic/biochemical response (CR) vs control and favorable safety. Patients with CHD (N = 150) were randomized 1:1:1: Arm A (control), no active anti-HDV treatment for 48W followed by BLV 10 mg for 96W (n = 51); Arms B and C, BLV 2 (n = 49) or 10 mg (n = 50), respectively, for 144W. All arms then entered a 96W treatment-free follow-up. Primary endpoint: CR (undetectable HDV RNA or decrease by ≥ 2 log10 IU/mL from baseline and ALT) at 48W. Other endpoints included viral response, biochemical response, and change in liver stiffness. Baseline characteristics: mean (SD) age, 41.8 (8.4) years; 57.3 % males; 82.7 % White; 47.3 % with compensated cirrhosis; 60 % on nucleos(t)ide analogues; mean (SD) HDV RNA, 5.05 (1.35) log10 IU/mL; mean (SD) ALT, 110.9 (69.0) U/L. CR was achieved by 22 (44.9 %) and 24 (48.0 %) patients in Arms B and C vs 1 (2.0 %) in Arm A (P < .0001). Viral and biochemical response rates were similar in both BLV arms and significantly greater than control at W48 (P < .0001). No adverse events led to BLV discontinu-

ation; no serious adverse events were attributed to BLV. Asymptomatic total serum bile salt elevations and injection-site reactions occurred more frequently with BLV 10 mg. BLV treatment resulted in a significantly greater CR vs control and was well-tolerated at W48.

P5.05 Relevance of microRNAs in SARS-CoV-2 infection of primary human hepatocytes

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DOI 10.1055/s-0042-1760043

Introduction Entry factors angiotensin converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) facilitate Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) entry into the host cells. Despite SARS-CoV-2's preference for respiratory system, extra-pulmonary organ involvement has been suggested. Recent studies report that SARS-CoV-2 leads to direct hepatic impairment in COVID-19 patients, necessitating further investigations about hepatic involvement. ACE2 and TMPRSS2 are expressed in primary human hepatocytes (PHH), suggesting a possible susceptibility to SARS-CoV-2. Despite this, data on infection and factors modulating functional regulation of SARS-CoV-2 infection in PHH are lacking. MicroRNAs (miRNAs) are approximately 22 nucleotide-long non-coding RNAs that have been shown to regulate various cellular processes including virus-host interactions. We aimed to study the susceptibility of PHH to SARS-CoV-2 and to evaluate the potential of miRNAs in modulating viral infection.

Materials and methods We investigated the role of miRNAs to regulate SARS-CoV-2 infection in PHH in vitro. To strengthen our findings, we analysed liver autopsies from COVID-19 patients.

Results We demonstrate that PHH can be readily infected with SARS-CoV-2, resulting in robust replication and sustained host responses as indicated by the upregulation of several interferon-stimulated genes. In silico analyses unravelled miR-200c-3p, miR-429 and miR-141-3p as candidate miRNAs targeting ACE2 and, let-7c-5p targeting TMPRSS2. Expression of these miRNAs reduced SARS-CoV-2 infection in PHH. Furthermore, expression of several endogenous miRNAs was altered upon SARS-CoV-2 infection in PHH and human liver autopsies.

Conclusion Our results show that PHH are susceptible towards SARS-CoV-2 and cellular miRNAs can diminish SARS-CoV-2 viral burden.

P5.06 CD103 identifies an ascites-resident NK cell subset with reduced effector capacity that originates in the intestine

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Introduction Liver cirrhosis is the end-stage of chronic liver disease and one main cause of decompensation and complicated course is the occurrence of ascites. However, presently less is known regarding the function and origin of NK cells in these patients, and particularly their role in the immune compartment "ascites". In this study, we aim to investigate the role of NK cells in the peritoneal cavity, a common anatomical site for infections in cirrhosis.

Methods Matched blood and ascites fluid were collected from 43 patients with decompensated liver cirrhosis without SBP and compared to healthy liver samples. Cytokines were measured using LUMINEX-based multiplex bead assay.

Results Total NK cells were elevated in the peritoneal cavity of patients with liver cirrhosis compared to matched circulating NK cells and displayed a more immature and hypofunctional phenotype with high expression of tissue-homing receptors. Indeed, expression of CD103 revealed an exclusive cluster of ascites-resident NK cells that is distinct from blood and liver NK cells. This was further corroborated by an increase of distinct pro-inflammatory cytokines in the ascites indicating intestinal permeability. Certainly, CD103 + NK cell frequencies correlated with these cytokines but not with disease severity suggesting a passive influx of this intestinal-derived NK cell subset due to decreased epithelial barrier function and the anatomic proximity of the intestine and peritoneal cavity.

Conclusion The ascites of patients with decompensated liver cirrhosis contains a unique ascites-resident CD103 + subset of NK cells with reduced effector capacity that may originate from the leaky gut observed in these patients.

P5.07 mRNA based expression of Hepatitis E specific neutralizing antibodies in liver cells as therapeutic option

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Hepatitis E virus (HEV) is a non-enveloped single-stranded RNA virus and the most common cause of acute hepatitis in humans worldwide. Serological marker studies showed that 420.000 individuals in Germany become infected with HEV annually. Chronic HEV infections can arise in approximately 50 % of infected immunosuppressed individuals. This can lead to rapid progression of liver fibrosis and cirrhosis with the ultimate need for liver transplantation. As no approved treatment for hepatitis E is currently available, there is an urgent need for effective antiviral therapeutics.

Neutralizing antibodies have been identified by analyzing memory B-cells from patients who have undergone HEV infection. These antibodies potently inhibit virus entry into the cell. The recent success of mRNA-based vaccines and therapeutic approaches has led to major innovations that have contributed to further development of mRNA vaccines and associated technologies. Previous studies in in vitro and in vivo models have shown that the expression of monoclonal antibodies can be induced by transfection of the corresponding mRNA into liver cells.

The aim of this project is to produce suitable mRNA to induce the most efficient production of anti-HEV specific antibodies in cell culture. For this purpose, we first established an mRNA production and transfection system by testing different signal peptides and 5'UTRs for protein expression levels. The sequences of the previously identified human monoclonal antibodies were inserted into the most efficient mRNA formulation. The production of functional antibodies in liver cell lines was confirmed by ELISA. Neutralization assays further confirmed the antiviral activity of the antibodies.

P5.08 Qualitiy of life-scores improve after 96 weeks of PEG-IFNa-2a treatment of hepatitis D: an analysis of the HIDIT-II trial

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Chronic Hepatitis D (CHD) is caused by co-infection with the hepatitis delta virus (HDV) of individuals with hepatitis B virus infection (HBV, CHB). It is considered the most severe form of viral hepatitis, promoting liver fibrosis, cirrhosis and hepatocellular carcinoma. Additionally, CHD has shown to cause worse patient related outcomes as compared to CHB alone. Until recently, only pegylated interferon based therapeutic schemes were available for treatment of CHD.

In this study we investigated the quality of life (QOL) as assessed by the Short Form 36 Health Survey (SF-36) of well compensated CHD patients during the HIDIT-II trial which investigated treatment of pegylated interferon alfa (PegINGa-2a) in combination with tenofovir disoproxil or placebo over a 96-week treatment period.

As expected, the overall QOL of our cohort was reduced in both physical and mental components as compared to a reference population. Interestingly, during PegIFNa-2a treatment, the decrease of QOL was lower than anticipated. About 45 % of the study cohort showed a minimal clinically significant decrease of the physical component score during treatment and this was unchanged 24 weeks after treatment. On the other hand, 50 % of patients showed a minimal clinically significant increase of the mental component score at the end of treatment. Compared to baseline, 24 weeks after the end of treatment, slight improvements in the QOL were observed.

Overall, our findings suggest a reasonable tolerance of PegIFNa-2a based treatment of CHD patients. Additionally, some CHD patients may benefit from off-treatment improvements in QOL.

P5.09 Treatment with Bulevirtide Improves Patient-reported Outcomes in Patients with Chronic Hepatitis Delta: An Exploratory Analysis of a Phase 3 Trial at 48 Weeks

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Bulevirtide (BLV), a novel NTCP entry inhibitor, is conditionally approved in the European Union for the treatment of chronic hepatitis delta (CHD). We report an exploratory analysis of quality-of-life (QoL) outcomes in patients with CHD after 48 weeks of treatment with BLV 2 mg in an ongoing Phase 3 trial.

MYR301 (NCT03852719; EudraCT 2019-001213-17) is a randomised, open-label, parallel-group, multicentre trial that assigned 150 CHD patients (1:1:1) to 3 exploratory arms (BLV 2 or 10 mg or control) for up to 3 years. Control patients received no active anti-HDV treatment until Week (W)48. Patients completed the Hepatitis Quality of Life Questionnaire (HQLQ) at study baseline (BL), W24, and W48. Higher scores on the HQLQ (range 0 – 100) indicate better health. Interim W24 results were reported previously.

BL characteristics were well balanced between BLV 2 mg (n = 49) and controls (n = 51). From BL–W48, BLV 2 mg was associated with improvements in all HQLQ domains; > 5-point improvements were observed for 10 of 14 items. Treatment differences vs controls in least-squares mean changes from BL–W48 were statistically significant (P < .05) for role–physical, hepatitis-specific (HS) limitations, and HS health distress. Improvements with BLV 2 mg seen at W24 were largely maintained or increased at W48.

CHD patients receiving BLV 2 mg showed improvements at W48 in all HQLQ domains, while patients in the control group remained largely unchanged, apart from improvements in health distress and HS health distress.

P5.10 Intra-host diversity of hepatitis E virus polymerase during sofosbuvir treatment

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Hepatitis E virus infections remain a serious problem in immunocompromised patients, leading to chronic infections in about 50 % of cases. Off-label use of Ribavirin is the only treatment option because no HEV-specific antivirals are available. Recently, the hepatitis C virus polymerase inhibitor Sofosbuvir was evaluated as a potential antiviral against HEV during a 24-week Sofosbuvir multicenter phase II pilot trial in nine patients (Cornberg et al. 2020).

In this study, we performed amplicon deep sequencing to determine viral dynamics during treatment and characterized high frequency variants that may affect susceptibility to Sofosbuvir using an HEV-based reporter cell culture system.

Viral diversity was at high levels in most patients, indicating a high capacity of the viral population to adapt to treatment-mediated selection pressure, which may have been associated with treatment-resistance variants. Amino acid substitution that had become dominant (A1343V, K1383N, D1384N, A1567V) during treatment were cloned into a HEV-3 replicon system to assess their effect on replication fitness and Sofosbuvir sensitivity. The half maximal effective concentration (EC50) was increased up to 10-fold above the control, suggesting that variants associated with lower susceptibility were selected during Sofosbuvir treatment.

To conclude, viral population dynamics play a critical role during antiviral treatment. High population diversity during Sofosbuvir treatment led to the selection of variants with higher fitness.

P5.11 The Interplay of antigen and antibody in HEV infection

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Although infection with the Hepatitis E Virus (HEV) is the most common cause of acute viral hepatitis worldwide, the serological response in HEV infected patients is not yet fully understood. Interestingly, immunocompromised individuals are often unable to clear the virus and develop chronic infection. During HEV infection, high amounts of glycosylated and non-infectious dimers of HEV's capsid protein (pORF2) are secreted. These dimers can be found in the serum even after clearance of the virus as determined by undetected HEV-RNA in stool and serum. We hypothesize that among other mechanisms, the lack of glycan sensitive antibodies not captured by these glycosylated forms of the capsid protein, contributes to a chronic disease course. We therefore aim to further investigate the interplay of antigen secretion and antibody detection over the course of HEV infections.

To this end, we established two enzyme linked immunosorbent assays (ELISA). First, we established an ELISA that detects anti-pORF-2-IgG in patients. To reach a higher specificity, we only use the protruding domain of pORF2 for detection, which is the target of neutralizing antibodies. Secondly, we established a sand-

wich-ELISA detecting pORF2 in patient sera. Importantly, this ELISA can differentiate between only non-glycosylated, infectious pORF2 and total pORF2 present. Ultimately, we want to correlate the course of antigen positivity and IgG response in different patients with different clinical courses of HEV infection.

This will lead to a better understanding of the interplay of antigen and antibody response in HEV infections and might identify determinants for the clinical outcome.

P5.12 Characterization of viral replication in different Hepatitis B virus-transgenic mice and its impact on antiviral signalling in vivo

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Background & Aims Pathogenesis of hepatitis B virus (HBV) infection is driven by innate and adaptive immunity. One aim of this study was to investigate how hepatitis B surface antigen (HBsAg) affects intrinsic antiviral signalling in HBV-transgenic mouse models that accumulate, lack or secrete the HBsAg. Another aim was to investigate how HBV HBsAg affects Kupffer cell populations in the different HBV-transgenic mouse strains.

Methods Liver tissue from C57/Bl6 and HBV-transgenic mouse strains (tg(Alb1HBV)44Bri (Alb/HBs), tg1.4HBV-s-mut and tg1.4HBV-s-rec [F1 generation of Alb/HBs × tg1.4HBV-s-mut]) was analysed using transmission electron microscopy (TEM) and confocal microscopy. HBV replication was characterized on RNA and protein level. Further F4/80 staining was performed in ECi cleared liver tissue and visualised by Light sheet fluorescence microscopy (LSFM). Responsiveness of parenchymal and non-parenchymal liver cells to poly(I:C) treatment in vivo was determined using quantitative RT-PCR and multiplex-based cytokine assay.

Results TEM visualised viral particles (Tg1.4HBV-s-rec), nuclear circular formations (Tg1.4HBV-s-mut and Tg1.4HBV-s-rec) and malformation of the endoplasmic reticulum (Alb/HBs). Confocal microscopy visualised HBsAg and HBcAg distribution in hepatocytes of HBV-transgenic mouse strains. Viral replication did not differ between Tg1.4HBV-s-rec and Tg1.4HBV-s-mut, except HBsAg levels. LSFM visualised a different intensity and volume of F4/80 positive cells in the liver of HBV transgenic mice. Cell type-specific and mouse strain-dependent interferon, cytokine, and chemokine expression were observed by LEGENDplex™ analysis.

Conclusion Liver cells of Tg1.4HBV-s-rec mice, which produce HBV particles and release HBsAg, exhibited a tolerogenic environment in vivo. Viral replication influences the distribution, size and intensity of F4/80 positive cells.

P5.13 Automated high-throughput image-based screen discovers members of the Akt serine/threonine kinase family as targets for treatment of HEV virus infection in vitro

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Forty-four years after the discovery of Hepatitis E virus as the etiological agent of viral hepatitis in human, treatment options remain limited to the off-label use of the nucleoside-analog ribavirin (RBV) and pegylated interferon-α. Although these drugs have made HEV infections manageable for the majority of patients, a considerable number of patients are either not eligible for standard treatment or do not respond to currently available treatment options. To find alternative antiviral drugs against HEV infections we developed a simple and effective image-based high-throughput screening assay using subgenomic HEV reporter replicons of genotype 3 expressing a GFP gene as a marker for viral replication in hepatoma cells. By screening up to 9,500 compounds derived from FDA-approved drug-libraries and performing dose-response assays with 170 of the most promising compounds, we identified at least 5 compounds that markedly inhibit viral infection at low micromolar concentrations in hepatoma cells. Finally, infection experiments with the human-derived HEV-3 p6-FL and the wild-boar HEV-3 83-2 virus identified inhibitors of the serine/threonine kinase Akt as a potential treatment target of pan-HEV infection in vitro.

In conclusion, screening drug-repurposing libraries proved to be a versatile tool for identifying novel drugs against HEV infections, but most importantly, our results suggest that pan-Akt inhibitors may be promising therapeutic candidates for the treatment of HEV infections.

P5.14 Liver and COVID-19 – Identification of early prognostic markers

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COVID-19 is a systemic disease affecting the liver to a crucial extent. This study investigates the impact of COVID-19 on the liver and possible early prognostic markers for the development of secondary sclerosing cholangitis (SSC). This rising complication in critically ill patients, also occurs after a severe COVID-19 infection. Early prediction for SSC has not been sufficiently investigated yet.

258 patients at intensive care unit (ICU) at the University Hospital of Regensburg were divided into groups depending on their medical history of preexisting liver diseases. Collected laboratory parameters during ICU comprised both baseline values, and worst values developed during hospitalization and were used to assess the rate of mortality between the different groups of patients. Development of SSC was evaluated against the baseline values.

Preexisting liver disease increased mortality rate from 39.3 % to 52.6 %. In both groups, mortality correlated significantly with an increase in liver values during ICU stay. High baseline values of Bilirubin (P = .002) or INR (P = .018) also correlated with mortality independently of preexisting liver diseases. The risk of developing SSC increased with high liver enzymes and correlated significantly (P = .004) with high AP levels at admission.

This study shows the high impact of COVID-19 on the liver and biliary system and possible complications in patients with and without preexisting liver diseases. Bilirubin and INR can be used as predictive factors regarding mortality. AP can be considered as an early predictor for development of SSC in patients with COVID-19 and could hint to optimized treatment strategies regarding ventilation and sedation.

P5.15 HEV genomic insertions identified in chronic patients enhance viral fitness

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The hepatitis E virus (HEV) is a long-neglected RNA virus and the major causative agent of acute viral hepatitis in humans worldwide with about 3 million

cases annually. HEV infections are usually self-limiting and asymptomatic in healthy individuals but can progress to chronicity and cause fulminant hepatitis in immunocompromised patients and other risk groups such as pregnant women. Recent data suggest that HEV has a very heterogeneous hypervariable region (HVR), tolerating major genomic rearrangements, which are mainly observed in chronic patients. We identified insertions in longitudinal samples of a ribavirin treatment failure patient, as well as in public databases and analyzed their influence on viral replication kinetics, virus production capacity and ribavirin sensitivity.

Noteworthy, all of the analyzed insertions contained a predicted nuclear localization sequence (NLS). The insertions were shown to increase the viral fitness, while not affecting the sensitivity towards ribavirin in the sub genomic replicon system. Furthermore, insertion-containing constructs produced higher viral titers than the parental HEV strain without insertion.

Besides, to the length polymorphism, distinct sequence patters up and downstream of the insertions were required for the observed effects. This study links insertions in the hepatitis E viral HVR to an increased replication capacity and identifies lysine residues as a common feature and determinant for fitness. Further investigation of the mechanism behind the replication advantage and the reason for the selection of those clones in vivo could reveal new therapeutic targets for the development of antiviral intervention strategies.

P5.16 HBV reactivation after start of bulevirtide mono-treatment for a HBV/HDV coinfected patient: a case report

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Background Bulevirtide is the first in-label treatment option for HBV/HDV coinfection in combination with nucleos(t)ide (NA) analogues or as monotherapy. We report a case of a patient with hepatitis B reactivation after start of bulevirtide monotreatment, which resulted in the need for a NA-start.

Case report A 45-y-old, male patient from Mongolia presented with HBV/HDV coinfection pretreated with interferon 2016. Baseline parameters were: HDV-RNA 73.500 IU/ml, HBV DNA not detectable, ALT 350U/l, elastography 10.6kPa/1,88 m/s with no clinical signs of livercirrhosis . After 4 months of bulevirtide monotreatment there was detectable HBV-DNA with 85 IU/ml, reaching 21.300 IU/ml with a concomitant ALT increase to 204 U/ml. Because of this HBV reactivation we started tenofovir (TDF) in addition to bulevirtide. HBV-DNA was non-detectable after one month of TDF. After one year of bulevirtide treatment ALT was normalized and elastography after 14 months of treatment showed a decrease of liverstiffness to 8kPa/1,65 m/s.

Discussion This is the first report of HBV reactivation in a patient who was negative for HBV-DNA without the need for NA-treatment before the start of a bulevirtide-monotherapy. Similar to the occasional HBV activation observed during DAA-treatment for HCV infection, monotherapy with bulevirtide in HBV/HDV-coinfected patients who are negative for HBV-DNA might pose a risk for HBV activation. The underlying mechanism for the observed HBV reaction is unclear. Frequent monitoring of respective patients for HBV reactivation is necessary.

P5.17 Changing HCV patient profiles: insights from a large multinational real-world sofosbuvir/velpatasvir (SOF/VEL) dataset

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Background and Aims More HCV-patients (pts) being started on direct-acting antivirals (DAAs) are belonging to vulnerable populations. This real-world analysis describes the profiles of these populations.

Method HCV-pts (18 + years [y]) from 37 clinical cohorts across nine countries treated with SOF/VEL for 12 weeks without ribavirin were included. Those with a history of decompensation or prior NS5A-inhibitor exposure were excluded. This descriptive analysis evaluated patient characteristics, time to treatment (TT [time from HCV-RNA diagnosis to DAA-initiation]), and sustained virological response ≥ 12 weeks after end of treatment (SVR), stratified by age and sex.

Results Among 6356 pts, 2274 were aged < 50y, 2568: 50 – 65y, and 1514 > 65y. The percentage of male was decreasing with age. Approx. 20 % of all pts had past IV drug use. Irrespective of age, male pts were more likely to

have compensated cirrhosis and HCV genotype (GT) 3 infection. Results in vulnerable male patients: Higher likelihood of incarceration in age-group ≤ 65y, significantly fewer mental health disorders and median TT was shorter.. Use of antipsychotics appeared similar, irrespective of sex.. In 5845 pts with valid result, SVR was high across age ranges, independent of sex (< 50y: female 98.7 %, male 99.2 %; 50 – 65y: female 98.7 %, male 98.1 %; > 65y: female 99.3 %, male 98.3 %). 475 pts did not achieve SVR for a non-virological reason; mostly loss to follow-up, independent of gender.

Conclusion Independent of gender, SOF/VEL results in high SVR rates. Significantly fewer mental health disorders were observed in male pts and TT was shorter.

P5.18 Anti-HEV seroprevalence varies largely in dogs, cats, and horses in Germany

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Background Hepatitis E virus (HEV) genotype 3 infections in Germany are mainly transmitted zoonotically by consumption of swine meat. Furthermore, there is evidence that pets might get in contact with HEV but the relevance of pets as possible hosts of HEV in Germany still needs to be defined.

Methods In this study 365 serum samples of pets (124 dogs, 119 cats, and 122 horses) have been tested for HEV by PCR and for anti-HEV antibodies by a commercial seroassays, designed for the use in mammals (MP-assay).

Results The anti-HEV seroprevalence determined by the MP-assay varied largely between dogs (10 %), cats (6 %), and horses (2 %). The difference between seroprevalence rates in dogs vs horses ($p = 0.01$) reached statistical significance (Chi-square test). Anti-HEV ELISA OD values were significantly higher in dogs in comparison to cats ($p = 0.008$) or horses ($p < 0.001$) and in cats compared to horses ($p = 0.008$, C, Mann-Whitney test). None of the pet serum samples tested PCR positive.

Conclusions This serological study suggests that dogs and eventually cats get infected with HEV in Germany with a relevant frequency, while horses are of minor relevance. Thus risk patients (e.g. transplant recipients) should be informed about dogs being a potential source of HEV infection.

P5.19 Polypharmacyand prevalence of multi drug-drug interactions in hepatitis C patients treated with pangenotypic direct acting antivirals: an analysis from three European countries

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DOI 10.1055/s-0042-1760057

Background Various guidelines recommend thorough drug–drug-interaction (DDI) evaluation before starting pangenotypic direct-acting antiviral (pDAA) therapy.

Studies have analyzed DDIs in HCV-patients receiving pDAAs and comedications only by pairwise interaction which does not reflect the polypharmacy in HCV-patients. This study aimed to evaluate the proportion of HCV-patients with multiple comedications causing potential DDIs in three European countries.

Method Prescription data of pDAA treated HCV-pts from Germany, Italy, and Spain were collected. DDIs were identified using Liverpool-database. Multi-DDI profile was defined as the use of ≥ 2 comedications each predicted with a DDI specific to the used pDAA . Potential DDIs were summarized as an increase in comedication exposure (safety impact) or a decrease in DAA exposure (efficacy impact).

Results 10,755 HCV-pts were treated with sofosbuvir/velpatasvir (n = 4583) or glecaprevir/pibrentasvir (n = 6172). Patients: 4950 German, 4185 Italian, and 1620 Spanish. 58.9 % received any comedication during pDAA therapy, and 20.2 % were at risk of DDI. Multi-DDI risk: 566 patients were taking ≥ 2 comedications, each with a potential DDI with their pDAA, representing 5.3 % of HCV-patients and 8.9 % receiving any comedication. Evaluation of predicted DDI impact: 23.9 % of patients taking ≥ 2 comedications risked efficacy impact, mainly due gastrointestinal drugs and analgesics. 26.5 % risked safety impact, mainly due cardiovascular and nervous system drugs.

Conclusion 8.9 % of analyzed pts taking medications during pDAA therapy are at risk of multi-DDIs. Accurate prediction of the impact on efficacy and safety is difficult. A thorough pDDI assessment before HCV therapy in comorbid patients is recommended.

P5.20 Intracellular “in silico microscopes” – fully 3D spatial Hepatitis C virus replication model simulations

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Virus pandemics and endemics cause enormous pain and economic, political, and social costs and turmoil. While the Covid19 pandemics induced obvious damages, the "silent" Hepatitis C virus (HCV) infection induced liver damages are the main reason for liver transplantations. HCV-generated virus genome replication factories are housed within virus-induced intracellular structures termed membranous webs (MW) which are derived from the Endoplasmatic Reticulum (ER). Up to now, very advanced experimental data such as highly spatially resolved fluorescence and electron-tomography data often do not enter computational HCV viral RNA (vRNA) cycle models. Based upon diffusion-reaction partial differential equation (PDE) models, we are developing fully 3D resolved “in silico microscopes” to mirror in vitro / in vivo experiments of the intracellular vRNA cycle dynamics. Our first models described the major components (vRNA, non-structural viral proteins - NSPs - and a host factor). The next steps incorporated additional parameters: Different aggregate states of vRNA and NSPs, and population dynamics inspired diffusion and reaction coefficients instead of multilinear ones. Our work in progress framework presently is merging effects restricted to 2D manifold surface grids (e.g. ER surface, NSP diffusion) with others occurring in 3D volume meshes (e.g. cytosol, host factor supply). We estimate and incorporate realistic parameters such as NSP diffusion constants. The simulations are performed upon experimental data based reconstructed cell geometries and help understanding the relation of form and function of virus replication. In the long run, our framework might help to facilitate the systematic development of efficient direct antiviral agents and vaccines.

P5.21 Strong decline of intrahepatic HDV markers and signs of liver inflammation after 48 weeks of treatment with Bulevirtide in chronic hepatitis D patients: Combined intrahepatic results from the clinical trials MYR203 and MYR301

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Background Bulevirtide (BLV, Hepcludex) is the only approved treatment for patients chronically infected with HDV in Europe. BLV is a first-in-class entry inhibitor blocking the HBV/HDV-receptor sodium taurocholate co-transporting polypeptide (NTCP).

Aim To investigate the antiviral efficacy of BLV treatment in paired liver biopsies obtained at baseline and after 48 weeks of treatment.

Methods We performed combined analyses of paired biopsies originating from the clinical trials MYR203 (phase 2) and MYR301 (phase 3). Patients with chronic hepatitis D were randomized to receive 48 weeks of BLV at 2mg or 10mg/day. Some patients received Tenofovir (TDF). A delayed treatment arm served as comparator. Virological parameters and infection-related host genes were assessed by qPCR and immunohistochemistry.

Results At week 48, intrahepatic HDV RNA declined with median reductions from baseline of 2.2Log10 with 2mg (n = 27) and 2.7Log10 with 10mg (n = 37), while HDV RNA levels did not change in the comparator arms (0.1Log10; n = 18). The reduction of HDAg positive cells determined in BLV-treated arms strongly correlated with the decrease determined by qPCR. Transcriptional levels of inflammatory chemokines (e.g. CXCL10) and interferon-stimulated genes (e.g. ISG15) concomitantly decreased in all BLV-treatment arms. High levels of total HBV RNA transcripts and low pgRNA loads were indicative of a highly transcriptional HBV DNA integration burden. BLV treatment reduced neither total HBV RNA nor pgRNA.

Conclusion We observed a strong dose-dependent decline of all intrahepatic HDV markers in BLV-treated patients. Additionally, intrahepatic HDV decline was associated with a decrease in inflammatory gene expression suggesting an improvement in liver inflammation.

P5.22 HBs-directed T cell engager antibodies foster efficient recruitment of T cells and lead to strong reduction of HBV infection in livers of humanized mice

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Background/Aim Recent studies have shown that bispecific T cell engager antibodies (TengAbs) binding HBV envelope proteins on infected hepatocytes and CD3 or CD28 on T cells, can activate T cells, leading to control of HBV infection in vitro and of tumor cell growth in vivo (Quitt et al. J Hepatol. 2021). The aim was to assess the antiviral efficacy of TengAbs on HBV infection in vivo in HBV-infected, human liver chimeric mice.

Methods HBV-infected humanized mice received PBMCs isolated from blood of healthy human volunteers at day 0 and 4 of treatment and 4 i.p. injections of TengAbs (1mg/kg body weight, every 3 days; n = 4) or PBS (n = 3).

Results Treatment of HBV-infected mice with TengAbs induced strong intrahepatic recruitment of human CD4 + and CD8 + T cells. Antibody treatment resulted in reduction of viremia (1.4-log), circulating HBsAg (0.69-log) and HBeAg (0.55-log) in only 11 days. Intrahepatic levels of HBV pregenomic (pg) RNA (0.7-log), HBV DNA (1.4-log) and cccDNA (0.9-log) were efficiently reduced. Histology and RNA ISH revealed reduced numbers of HBcAg + cells and substantially lower expression levels of pgRNA in infected hepatocytes. These changes were accompanied by a transient increase of ALT (day 7), increased expression of granzyme B, IFN-γ and TNF-α, and a reduction in human serum albumin.

Discussion Treatment of HBV-infected humanized mice with bispecific T cell engager antibodies after transfer of human PBMCs efficiently reduced HBV viral load in vivo, demonstrating the efficient recognition of infected hepatocytes, and induction of both cytolytic and cytokine-mediated HBV-specific T cell immunity.

P5.23 The role of CXCR6 + γδ T cells in chronic hepatitis B virus (HBV) infection

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Current treatment options for chronic HBV infection can suppress HBV DNA and decelerate disease progression, but cannot cure HBV infection, defined as HBsAg loss. Immune responses are considered important for HBV pathogenesis and viral control or elimination. Human γδ T cells have been shown to play a role in various infectious diseases and are enriched in solid tissues, e.g. the liver, which may suggest a role in liver-associated diseases. However, their role in chronic HBV infection remains largely unclear. Therefore, we investigated the phenotype, function, and T cell receptor (TCR) repertoire of γδ T cells in patients with chronic HBV infection with different viral and clinical characteristics, including patients discontinuing long-term NA therapy.

We observed increased frequencies of γδ T cells in chronic HBV patients with high HB core related antigen level. Furthermore, these patients had a higher frequency of CXCR6 + γδ T cells, suggesting recruitment of those T cells to the liver. In line with this, analysis of liver tissue samples showed a higher frequency of CXCR6 + γδ T cells in the liver compared to the blood.

A pilot study showed that patients who experienced an increase in CXCR6 + γδ T cells during the ALT-flare after discontinuation of NA treatment later showed a trend towards HBsAg loss. Additionally, those patients showed a similar γδ TCR diversity.

In conclusion, our data indicate that CXCR6 + γδ T cells may be involved in disease pathogenesis in chronically infected HBV patients but may have antiviral features that may support functional cure.

P5.24 Targeting epigenetic imprints to modulate HBV-specific T cell responses

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Worldwide, over 296 million people have been diagnosed with chronic HBV (CHB) requiring lifelong therapy to reduce disease progression. However, functional cure of CHB (defined by loss of HBsAg) is rarely achieved and emphasizes the need of new therapeutic approaches. Hallmarks of chronic diseases are an impaired immune system and the presence of exhausted T cells with a distinct epigenetic signature. There are several concepts to improve their function, e.g. checkpoint inhibition via αPDL1. Unfortunately, αPDL1 cannot remodel exhaustion-associated epigenetic patterns.

We aim to identify molecular and epigenetic predictors of treatment responsiveness by investigating epigenetic signatures of immune cells in CHB patients. Additionally, we aim to improve the effect of αPDL1 by targeting epigenetic mechanisms.

Preliminary DNA methylation data suggest an increased biological age (predicted by epigenetic age) in CHB. Additionally, the age acceleration correlates with liver stiffness and an elevated CD8 + T cell population demonstrating a link between epigenetic changes in CHB patients, clinically relevant markers and immune cell populations.

To improve checkpoint inhibition, we performed 10-day expansion culture with peripheral blood mononuclear cells (PBMC). PBMCs were stimulated with HBV core overlapping peptide pool and treated with a combination of αPDL1 and DNA methyltransferase inhibitor (DNMTi). We observed cytotoxicity of high dose DNMTi on PBMCs. Additionally, some patients had an improved IFNγ + response of CD4 + and CD8 + T cells after pretreatment with DNMTi followed by aPDL1 treatment at day 3 post-stimulation.

The results indicate that epigenetic signature of T cells might play an important role in CHB immune response.

P5.25 Vaccine to Inhibit Autochthonous Transmission of Hepatitis (VaccinATE)

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Introduction and aim Hepatitis E virus (HEV) is the most common cause of acute viral hepatitis worldwide. In Germany, genotype 3 (GT3) is most prevalent and pigs are the main reservoir of this genotype. HEV GT3 is mainly transmitted to humans through consumption of contaminated meat products. Therefore, vaccination of pigs against HEV could consequently prevent the transmission of HEV to humans. Here, we aimed to identify the most suitable immunogen and immunization strategy for vaccination against HEV.

Methods We selected eight different protein- or DNA-based HEV vaccines to compare their efficacy, using rabbits as antibody donors. Antibody responses of the animals were analyzed for binding of HEV by means of ELISA and immunofluorescence. Furthermore, the antibody was functionally assessed utilizing neutralization assays. The avidity of antibodies from rabbit sera to the HEV antigen was measured using a urea-based ELISA.

Results and conclusion We identified that vaccination of rabbits with all different vaccines elicited anti-HEV IgG antibodies. All the antibodies have neutralization activity against HEV GT3. Furthermore, using immunofluorescence staining, we revealed that antibodies in the rabbit sera bind to HEV transfected HepG2 cells. According to the results of our urea-based ELISA, the anti-HEV IgG antibodies from inoculated rabbits had different avidity indices depending on the type of vaccine used. In this project, we could evaluate different vaccination technologies against HEV in rabbits. Based on our findings, the most suitable immunogens have been identified, paving the way for future studies on the immunization of pigs.

P5.26 Single cell analysis of HCV and heterologous virus-specific CD8 + T cell responses in patients with chronic hepatitis C before and after HCV elimination

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Background Chronic hepatitis C virus (HCV) infection can be cured in > 95 % of patients with direct-acting antiviral therapy (DAA). Several studies have shown that even in the long-term after viral elimination, a molecular scar remains in the HCV-specific immune response. The question arises whether HCV clearance affects immune responses against unrelated infections. The aim of this study was to investigate antigen-specific CD8 + T-cell responses against adenovirus, cytomegalovirus (CMV), Epstein-Bar virus (EBV), and hepatitis B virus (HBV) in DAA-treated, chronically HCV-infected patients with anti-HBc + .

Patients & Method Eleven HLA-A2 + , anti-HBc + patients with chronic HCV infection were included. Virus-specific CD8 + T-cells (adenovirus-E1A19, CMV-pp65495, EBV-BMLF1280, HBVcore18, HBVpolymerase455, HCV-NS31073, HCV-NS31406) were analyzed at therapy start, end of treatment and up to four years post treatment, using the BD Rhapsody single cell-system (transcriptome and combined T-cell receptor sequencing).

Results Virus-specific T-cells could be distinguished in six clusters based on its transcriptome. Heterogeneity was detected between all analyzed virus-specific responses, differently distributed across the transcriptomic clusters. Of note, this variation is even detectable within a single virus-specific response, particularly for HCV (comparing HCV-NS31073 and HCV-NS31406) and HBV responses (HBVcore18 and HBVpolymerase455), when comparing multiple epitope-specific responses. Moreover, longitudinal analysis of epitope-specific responses within one patient revealed that some virus-specific responses changed, while others stayed stable over time. Individual clonotype tracing revealed a skewed distribution over the six different clusters of the transcriptomic map.

Conclusion Our results suggest that DAA therapy has an effect that extends beyond the HCV-specific T-cell immune response and influences also other virus-specific responses.

P5.27 The risk-variant rs56258221 at the BACH2-locus associates with skewed polarization of naive CD4 + T cells towards pro-inflammatory phenotypes in primary sclerosing cholangitis

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DOI 10.1055/s-0042-1760065

Background and Aims The pathogenesis of Primary sclerosing cholangitis (PSC) is still unknown. Intrahepatic naive-like T cell population prone to polarize towards TH17 phenotype and several polymorphisms in immune-related genes has been linked to PSC. We hypothesized that genetic predisposition contributing to T cell phenotype in patients with PSC.

Methods Patients with PSC (n = 270) were genotyped for the disease-associated risk variants rs56258221 (BACH2), rs80060485 (FOXP1), rs4147359 (IL-2RA) and rs7426056 (CD28). T cell function and phenotype, in vitro polarization and proliferation, microRNA-assays, western blots and single-cell RNA sequencing was performed.

Results Functional in vitro experiments with naive CD4 + T cells from patients with PSC and healthy donors (HD) as controls showed increased capacity of PSC-derived cells to convert into pro-inflammatory T Helper 1 (TH1, 50.7 % vs. 42.9 %, p = 0.027) and T Helper 17 (TH17, 5,5 % vs. 2,2 %, p = 0.042) subsets. Moreover, lower conversion rate into induced regulatory T cells (iTREG, 9.6 % vs. 17.3 %, p = 0.022) could be detected. The observed effects were increased in rs56258221 (BACH2) carriers and not seen for the other variants in immune-related genes assessed. Interestingly, single-cell RNA sequencing of the T cell compartment identified a composition skewed towards activated phenotypes in rs56258221-carriers.Reduction of BACH2 on protein level was linked to a strongly increased expression of microRNA 4464, previously imputed to inhibit translation of BACH2.

Conclusion We here present comprehensive data linking the risk variant rs56258221 to the recently described dysregulated T cell phenotype in patients with PSC.

P5.28 Characterisation of HEV particles in serum, urine, ejaculate and stool using a linear gradient ultracentrifugation technique

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Background/Aim Hepatitis E Virus (HEV) particles exist in distinct forms: non-enveloped infectious particles as commonly found in faeces and less well-characterized quasi-enveloped particles in blood with less clear function. We aimed to optimize characterization of the distinct HEV particles produced and detected upon infection in vivo.

Methods Serum, urine, ejaculate and stool samples from HEV-positive patients and faeces samples from infected humanized mice were processed using a linear iodixanol-sucrose gradient. Fractions were collected, density determined

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by refractometry and tested by PCR for HEV RNA. Immunoblotting was performed to assess extracellular vesicles (EV) markers.

Results The presence of quasi-enveloped HEV particles (density 1.12-1.15g/cm3) was determined by qPCR in serum, urine and ejaculate of patients, while naked particles (1.23-1.26g/cm3) were found only in stool samples. Electron microscopy showed a diameter of non-enveloped HEV at ~34nm and of quasi-enveloped virion at ~46nm. EV marker proteins (e.g. FLOT1) were detected by immunoblot in serum-, urine- and ejaculate-derived fractions (densities 1.12-1.15g/cm3) but not in stool fractions. Intriguingly, faeces from infected humanized mice revealed a peak density between 1.16-1.19g/cm3. In line with human-derived stool samples, these fractions showed no detection of FLOT1 and had a similar particle diameter.

Conclusion The distribution of HEV quasi-enveloped and "naked" particles revealed that patient-derived urine and ejaculate samples are quasi-enveloped and therefore likely less infectious comparing to HEV-positive stool samples. Preliminary results suggest that the quasi-envelopes are spiked with EV marker proteins. The density difference determined between HEV particles originating from patient and mouse stool needs further investigation.

P5.29 When the pathologist is not too late (for once) – rare infectious causes of acute hepatic dysfunction with distinct histopathological features.

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DOI 10.1055/s-0042-1760067

Background Hepatic dysfunction of unknown cause is a major challenge for both treating physicians as well as pathologists involved in the diagnostic workup. The acute presentation of these patients and the broad spectrum of differential diagnoses remain a challenge. Core liver biopsy is a key diagnostic tool in evaluating acute hepatic dysfunction of unknown cause and may help to differentiate between infectious and non-infectious causes.

Material and Methods Retrospective cases of core liver biopsies in patients with hepatic dysfunction of unknown cause and laboratory confirmed infection at University Hospital rechts der Isar; Munich, were evaluated regarding histopathological criteria of infection. Both common and rare infectious causes of acute hepatic dysfunction were identified in this patient cohort. Overall, six cases with confirmed infection with Hepatitis A, Herpes simplex Virus, Adenovirus, and Coxiella burnettii were included.

Results Certain infections leading to hepatic dysfunction result in distinct histopathological changes that can be identified by the trained pathologist. Knowledge of these changes can aid in identifying infections as causes of hepatic dysfunction, in particular when blood tests for rare infectious causes have not yet been ordered by the treating physicians. Therefore, the pathologist may be able to initiate further confirmatory laboratory testing and definitive therapy in these medical emergencies.

P5.30 Inflammatory profiles are different between patients with EASL-CLIF- versus APASL-acute-on-chronic liver failure

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Background Definitions of acute-on-chronic liver failure (ACLF) are heterogeneous. Whereas extrahepatic organ failures play a central role in the EASL-CLIF- and NACSELD-definitions of ACLF, the APASL-definition focuses on the liver as most important organ failure. In the present study, we therefore determined associations between inflammatory molecules, presence and absence of ACLF, clinical parameters and outcome.

Methods Hospitalized patients with liver cirrhosis (N = 208) with or without ACLF were recruited from a prospective cohort study. 76 inflammatory molecules were quantified by proximity extension analysis assay (Olink, Uppsala, Sweden). Associations between inflammatory profiles and types of ACLF were determined.

Results Of 208 patients, 127 had no ACLF, while 81 had any ACLF. Of patients with ACLF, 17 had ACLF exclusively based on the APASL-definition, while 30 had ACLF exclusively based on the EASL-CLIF-definition. All 12 patients with NASCELD-ACLF also fulfilled the EASL-CLIF criteria. A differential secretion of inflammatory molecules according to the type of ACLF was observed. Overall, patients with APASL-ACLF (but without EASL/NASCELD-ACLF) had rather moderate changes of inflammatory mediators compared to patients with acute decompensation without ACLF, whereas patients who met the EASL- or NASCELD-definition of ACLF showed signatures of substantial systemic inflammation. Furthermore, a differential increase of mediators between EASL-CLIF- and APASL-CLIF-ACLF was observed, as for example FGF-19 and HGF were particularly increased in APASL-ACLF while VEGFA, FGF-23, TNF-beta or IL-17 are significantly upregulated in EASL-CLIF.

Conclusion Patients with APASL- versus EASL-CLIF-ACLF have partially distinct inflammatory profiles, which may point towards distinct pathophysiological mechanisms in different types of ACLF.

P5.31 The effect of testosterone on human T cells in health and autoimmune liver disease

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Females are more prone to develop autoimmune diseases, including primary biliary cholangitis (PBC). The mechanisms underlying this dichotomy are still unknown.

We aimed to unravel the effects of testosterone on human T cell phenotype and function, and how this may contribute to the pathogenesis of PBC. In order to investigate the direct and indirect effects of testosterone on T cells we analyzed two unique clinical cohorts: I) Female and male PBC patients compared to age and sex matched controls and II) transgender men receiving gender-affirming testosterone treatment. Ex vivo multi-color flow cytometry immunophenotyping, in vitro conversion assays and CITE-sequencing were used to characterize T cells and to identify changes upon in vivo testosterone treatment.

We found that female PBC patients showed increased numbers of TH1 cells and reduced ratio of Tregs to TH17 cells when compared to healthy controls. Importantly, in vitro conversion of naïve CD4 T cells into TH1 cells was significantly increased in female PBC patients further supporting the shift towards proinflammatory immune responses. Interestingly, female PBC patients presented with lower serum testosterone levels compared to healthy controls. Confirming an effect of testosterone on T cells, in the transgender cohort, we identified significant changes in T cells after high dose testosterone treatment. Thus, CD4 as well as CD8 T cell subset composition were affected by testosterone treatment. Together these data suggest that testosterone has a regulatory role in T cells in health and autoimmunity and that reduced androgen signaling may contribute to the pathophysiology of autoimmune liver diseases.

P5.32 In vivo labeling of nascent viral HBV and HDV RNA for investigating viral replication and clearance kinetics

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DOI 10.1055/s-0042-1760070

Background/Aim Labeling of newly synthesized RNA is a promising approach to investigate viral replication kinetics and stability of viral components in circulation. We aimed to label nascent host and viral transcripts in vivo to gain insights into replication dynamics and stability of HBV and HDV.

Methods Stably HBV/HDV infected humanized mice and animals treated with Bulevirtide (BLV) received a single injection of 2mg ethynyluridine (EU). Blood and livers were analyzed between 4h to 48h after EU administration. EU-labeled RNA was isolated using biotinylisation and binding to streptavidin-coated magnetic beads (click detection). Viral and host RNAs were analyzed by qRT-PCR and intrahepatic EU-labelling was visualized by staining.

Results Highest intrahepatic RNA labelling was visualized after 6h. Quantification of labeled RNA in liver revealed different host gene kinetics, with ISG15 peaking at 6h and housekeeper RNAs (GAPDH & RPL30) displaying slower decrease kinetics. HDV RNA showed a slower turnover compared with host and HBV RNAs in livers of co-infected mice. In serum, the decay of labeled HDV RNA also appeared slower compared to labeled pgRNA. Notably, HDV decay appeared further relented in BLV-treated mice.

Conclusion EU administration in HBV/HDV infected humanized mice enabled time-dependent labeling of nascent HBV- and HDV-RNA in liver and serum. These experiments indicate that not only host genes, but also HBV and HDV RNAs display distinct kinetics of turnover in liver and in circulation. The slight delay of HDV seroclearance in mice receiving BLV suggests that the drug may hinder NTCP-mediated uptake of HDV in the liver.

P5.33 Intrahepatically activated and antigen-selected B cells are bystanders, not drivers of autoantigen-driven hepatitis in mice

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Background Autoimmune hepatitis (AIH) is considered driven by an autoimmune CD4 + T cell response to hepatocellular antigens. B cells are also present in AIH livers, but it is not clear whether autoreactive B cells actively promote liver inflammation or are merely bystanders of T cell-driven pathogenesis.

Methods The B cell response in autoantigen-driven hepatitis was studied in the Alb-iGP_Smarta mouse model in which recognition of the GP model antigen in hepatocytes by CD4 + T cells causes spontaneous AIH-like disease (Preti M et al. JCI Insight 2021; 6:e141462).

Results T cell-driven experimental AIH in Alb-iGP_Smarta mice was marked by hepatic infiltration of plasma cells and B cells, particularly of isotype-switched memory B cells, indicating antigen-driven selection and activation. Immunosequencing of B cell receptor repertoires confirmed B cell expansion selectively in the liver, which was most likely driven by the hepatic GP model antigen. However, intrahepatic B cells did not produce increased levels of cytokines and their depletion with anti-CD20 antibody did not alter the CD4 + T cell response in Alb-iGP_Smarta mice. Moreover, B cell depletion did not prevent spontaneous liver inflammation and AIH-like disease in Alb-iGP_Smarta mice.

Conclusions Selection and isotype-switch of liver-infiltrating B cells occurred in the presence of CD4 + T cells recognizing liver antigen. However, recognition of hepatic antigen by CD4 + T cells and CD4 + T cell-mediated hepatitis was not dependent on B cells. Thus, B cells in AIH livers need not be drivers of liver inflammation, but could be bystanders.

P5.34 Association of a TLR7 variant with spontaneous clearance of hepatitis B virus infection in Caucasians

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Background Toll-like receptor 7 (TLR7) is a major part of the innate immune system generating immune responses against hepatitis B virus (HBV). Recently, TLR7 activation has been shown a promising target for the treatment of chronic hepatitis B. However, polymorphisms within the TLR7 gene might alter immune responses and therapy efficiency. Therefore, we investigated the impact of the TLR7 polymorphisms rs179008, rs864058 and rs2302267 on the course of HBV infection.

Method In this study, 530 Caucasian patients with chronic hepatitis B virus infection and 195 individuals with spontaneous HBV surface antigen (HBsAg) seroclearance (SC) were enrolled. Genotyping of the TLR7 SNPs was performed. Serum cytokine levels were measured in 195 patient samples.

Results The genotype distribution of the TLR7 SNP rs179008 was significantly different between patients with chronic HBV infection and HBsAg SC ($p = 0.003$). The AT/TT genotypes were significantly associated with an increased likelihood of HBsAg SC in adjusted forward logistic regression analysis (OR = 1.48 [95 % CI: 1.01-2.17] $p = 0.045$). There were no significant differences found for the other TLR7 SNPs. Carriers of the rs179008 AT/TT genotypes with HBsAg SC showed lower serum levels of IL-1 beta ($p = 0.03$), TNF alpha ($p = 0.01$), IFN alpha ($p = 0.0008$), IFN beta ($p = 0.019$) and IL-10 ($p = 0.029$) than carriers of the AA genotype.

Conclusion We confirmed the previously reported association of the TLR7 rs179008 variant with HBsAg seroclearance in a Caucasian cohort and the effect on cytokine production. Our findings further strengthen the relevance of the genetic predisposition in individualized medicine and the development of new TLR7-directed therapies.

P5.35 Association of variants of interleukin 10 and 12A with the progression of hepatitis B virus infection in Caucasians

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Background and Aims Interleukin (IL) 10 and IL-12 contribute to immune responses against hepatitis B virus (HBV). Polymorphisms in the IL-10 and IL-12A genes might affect the clinical outcome of HBV infection. We evaluated the association of IL-10 rs1800896 and rs3024490 and IL-12A rs568408 and

rs2243115 with the progression of HBV infection and development of severe liver disease stages in a Caucasian population.

Method 636 Caucasian patients with chronic hepatitis B (CHB), 239 individuals with spontaneous HBV surface antigen (HBsAg) seroconversion (SC) and 254 healthy controls were enrolled. The CHB group included 255 patients with hepatitis B e antigen (HBeAg)-negative (CHB), 99 patients with HBeAg-positive CHB and with HBeAg-negative HBV infection (inactive carriers, IC) (n = 228). 104 CHB patients had liver cirrhosis. Serum levels of cytokines were retrospectively measured in serum samples derived from patients with HBV infection (n = 195) or in in healthy controls (n = 160).

Results In adjusted multivariate analysis, the IL-10 rs1800896 AG/GG genotypes were significantly associated with an increased probability of HBsAg SC (OR = 1.75 [95 % CI: 1.04-2.94], p = 0.034), with an increased likelihood of IC (OR = 1.93 [95 % CI: 1.05-3.54], p = 0.034) and with increased serum cytokines levels in female patients. In contrast, the IL-12A rs568408 AG/AA genotypes were independently associated with an increased risk to develop liver cirrhosis with an OR of 1.90 (95 % CI: 1.07-3.39, p = 0.029) in male patients.

Conclusion The current study shows a sex-related association of the IL-10 SNP rs1800896 and IL-12A SNP rs568408 with different stages of HBV infection and with HBV-related liver cirrhosis in Caucasian patients.

P5.36 Comparative analysis and distribution of distinct HBV particles and antigens in serum of patients and human liver chimeric mice according to density

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DOI 10.1055/s-0042-1760074

Background The characterization of viral and subviral particles (SVPs) circulating in HBV-infected patients and their possible association with extracellular vesicle (EV) markers is still poorly defined.

Aim To characterize distinct types of HBV particles, viral antigens and EV markers in serum of chronic hepatitis B patients and in humanized mice as surrogate model of HBV infection.

Methods Serum samples of treatment naïve HBV-positive patients and humanized mice were separated using iodixanol sucrose gradients optimized for the density between 1.10 and 1.22g/cm³. Fractions were quantitatively analysed for HBV DNA, RNA (pgRNA and subgenomic RNAs), HBsAg and HBeAg. Western Blot was performed to assess EV markers.

Results HBV infected mice and patients showed the main peak for HBsAg in fractions with lower densities between 1.13 to 1.17g/cm³ and HBeAg between 1.12 and 1.18g/cm³. Interestingly, the EV marker FLOT1 was also exclusively found in fractions with a density of 1.1-1.15g/cm³, indicating an overlap of the EV fraction with SVPs and HBeAg. HBV DNA and HBV RNAs were detected predominantly at 1.17-1.20g/cm³. The density distribution of viral nucleic acids and antigens between humanized mice and patient derived samples appear similar. The presence of two distinct peaks at 1.17-1.20g/cm might be associated with virions and non-enveloped core particles containing either DNA or RNA.

Conclusion These preliminary analyses indicated that the distribution of circulating HBV particles and antigens released from infected human and mouse livers are similar. The distribution of distinct hepatitis Delta viral particles is currently under investigation and results will be presented.

P5.37 Distinct profiles and kinetics of soluble inflammatory markers in patients with acute and chronic HCV-infections and SARS-CoV-2-infections: Implications for the long-term consequences of viral infections?

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DOI 10.1055/s-0042-1760075

Abstract

Background and Aims Viral infections occur acutely but can also progress chronically, with the immune system having a central role in immunopathogenesis. The question arises whether all alterations in immune responses are reversible after viral elimination (spontaneously or by therapy). Therefore, the aim of this study is to compare soluble inflammatory markers (SIM) during and after infection with SARS-CoV-2 and acute and chronic HCV-infections.

Patients and Method Patients with acute HCV (n = 29), chronic HCV (n = 54), SARS-CoV-2 (n = 39) and 31 healthy-controls were included. Blood samples were tested at baseline, end of treatment/infection, and follow-up (≥ 9 months after baseline). IL-12p70, IL-1b, IL-4, IL-5, IL-6, IL-8, TNF, IFN-g, IL-10, IL-22, CXCL-10, MCP-1, MIP-1b, ITAC were quantified using the HD-SP-X Imaging and Analysis SystemTM.

Results SIM profiles in patients with acute HCV were substantially elevated at baseline and the decrease during follow-up was considerably less compared to the SARS-CoV-2 cohort. In chronic HCV-patients, viral elimination by therapy resulted in a decrease in SIM, although not always to those of controls. Cirrhotic HCV patients had higher SIM levels after HCV elimination than non-cirrhotic chronic HCV-patients. In the SARS-CoV-2 cohort, most SIM returned to levels of controls 3 months after baseline.

Conclusions SIM profiles and kinetics after viral elimination differ between blood-borne acute and chronic HCV- and respiratory SARS-CoV-2-infections. The immunologic imprint 9 months after cured HCV-infection (both acute and chronic) appears to be more pronounced than after SARS-CoV-2-infection. Further analysis is needed to correlate the SIM profile with the clinical phenotype (long-HepC vs. long-COVID-19).

P5.38 Mucosal-associated invariant T cell are rendered dysfunctional within the tumour microenvironment in HCC in a cell-contact dependent manner

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Hepatocellular carcinoma (HCC) is a major cause of cancer deaths worldwide and response rates to immunotherapy remain low. MAIT cells, innate-like T cells enriched in the liver, express cytolytic molecules and have therefore been attributed anti-tumour properties. In this study, we aim to decipher direct interactions between MAIT cells and HCC cells in order to unravel mechanisms of immune cell dysfunction within the tumour microenvironment in HCC.

MAIT cells were isolated from tumour or adjacent liver tissue of human patients with HCC, other liver tumours or healthy controls. MAIT cells were co-cultured with HCC cell lines in vitro, MAIT cell phenotype and function was analysed by multi-colour flow cytometry.

We show that MAIT cells frequency is significantly and specifically reduced in HCC tumour tissue compared to adjacent liver tissue. Tumour-educated MAIT cells showed high exhaustion marker expression and significantly impaired effector function, suggesting MAIT cell dysfunction within the tumour microenvironment in HCC. We studied the direct interaction between HCC cells and MAIT cells in a co-culture system using MAIT cells and HCC cell lines or primary hepatocytes. We observed a significant downregulation of effector molecule expression by MAIT cells in presence of HCC cells, but not hepatocytes, which translated into impaired killing capacity towards these HCC cell lines. Mechanistically, induction of MAIT cell dysfunction by HCC cells was dependent on direct cell-cell contact.

Taken together, we show that MAIT cells are rendered dysfunctional within the HCC microenvironment, suggesting MAIT cells as a potential target for novel anti-cancer therapies in HCC.

P5.38 Heterogeneity of hepatitis B virus (HBV) basal core promotor (BCP) variants during treatment with peginterferon alfa-2a is associated with low HBV replication

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The presence of precore (PC) and BCP variants is associated with HBeAg seroconversion (SC) during treatment with PEG-IFN, but the mechanism of HBeAg SC is still unknown. We investigated the occurrence of BCP/PC variants during treatment with PEG-IFN and their association with replication and treatment response. Therefore, patients with HBeAg positive chronic hepatitis B receiving PEG-IFN for 48 weeks (39 with and 28 without HBeAg SC until week 72) were retrospectively analysed. Viral markers (HBeAg, HBcrAg, HBsAg, HBV DNA and HBV RNA) were quantified and BCP (A1762T/G1764A) and PC stop (G1896A) variants were analysed by direct sequencing on HBV DNA basis at every time point. Results revealed that in patients with HBeAg SC, BCP/PC variants were present in 23/39 (60.5 %) patients before treatment and in 37/39 (94 %) patients at week 72. In contrast, only 7/29 (24.1 %) patients without HBeAg SC showed a mutation before treatment and additional 7 patients developed BCP/PC variants until week 72 (48 %, p = 0.003). BCP/PC variants were strongly associated with decreasing HBV DNA, HBV RNA and HBeAg, but not HBsAg and HBcrAg levels in both patient groups. Moreover, the occurrence of BCP/PC variants was associated with low HBV RNA levels and vice versa, independent of the treatment response. In conclusion, we found higher frequencies of BCP/PC variants in patients with HBeAg SC as compared to patients not achieving HBeAg SC. However, irrespective of HBeAg SC, the occurrence of these variants leads to a decrease in HBV replication as measured by undetectable HBV RNA and abolished HBeAg production.

P5.39 A Case of Fulminant Hepatitis B Reactivation After Hepatitis C Treatment in Hepatitis B + C Co-infection

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Objective Replicative activity of both viruses is lower in hepatitis B + C coinfection. In 2-10 % of anti-HCV + patients, HbsAg may be positive. Reactivation due to both interferon + direct antiviral therapy has been reported in HBV + HCV coinfection in the literature. Fulminant after hepatitis C treatment We aimed to present a case who developed hepatitis B and referred to liver transplantation

Case A 52-year-old male patient was started on glicepravir + pibrentasvir treatment for non-cirrhotic hepatitis C in an external center 4 months ago and applied to our outpatient gastroenterology outpatient clinic with the complaints of jaundice and mild disorientation 2 months after the end of the treatment. oriented, marked icteric. TA: 120/70 pulse: 90 fever: 36.5 C, saturation was 96 %. Laboratory values ALT:1500 IU, AST:2000 IU, ALP:180 IU,GGT:50 IU, Total bilirubin:15 mg/dl, direct bilirubin: 7 mg/dl INR: 2. It was HbsAg + , Anti Hbs-, HbeAg -, AntiHbc Ig G + , Anti HCV + . HCV RNA and HBV DNA were sent. HBV DNA values of the patient were not checked before hepatitis C treatment. His HBV DNA value was 2.83x 10 * 3. He had Grade 1 Hepatic encephalopathy and mild disorientation. Fulminant hepatitis B reactivation was considered with the current findings and laboratory values. and liver transplantation of the patient was performed in the upper center. No problems were encountered in the post-transplant follow-up of the patient

Conclusion Every patient for whom hepatitis C treatment is planned should be screened for hepatitis B, According to her, if necessary, the two viruses should be treated TASL.

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