Thieme

40. Jahrestagung der Deutschen Arbeitsgemeinschaft zum Studium der Leber

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26.–27. Januar 2024, Haus der Technik e.V., Essen

Tagungspräsident:
Univ.-Prof. Dr. Ulf Peter Neumann

Lecture Session I BASIC HEPATOLOGY (FIBROGENESIS, NPC, TRANSPORT) 26/01/2024, 13.10pm–13.55pm, Lecture Hall

L1.01 Inhibition of the Renal Apical Sodium-Dependent Bile Acid Transporter Prevents Cholemic Nephropathy in Bile Duct ligated Mice

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Cholemic nephropathy (CN) is a severe complication of cholestasis-associated liver diseases, and no specific treatment is available. To understand the driving mechanism and identify therapeutic strategies, obstructive cholestasis was induced by bile duct ligation (BDL) in mice; bile flux in kidneys and livers was visualized by intravital imaging, supported by MALDI-MSI and LC-MS/MS. We show that proximal tubular epithelial cells (TEC) reabsorbed and enriched BA after BDL, leading to oxidative stress and death of proximal TEC, casts in distal tubules and collecting ducts, damage and leakiness of peritubular capillaries, and glomerular cysts. Since TEC express the apical sodium-dependent bile acid transporter (ASBT) at their luminal membrane, we used the novel compound AS0369, a systemically bioavailable ASBT inhibitor, to block BA uptake. Inhibition of the renal ASBT almost completely prevented kidney injury up to 6 weeks after BDL. Similar results were obtained in mice with humanized BA spectrum. To check the translational relevance, we analyzed serum BA, bilirubin, and kidney injury molecule (KIM-1) in patients with advanced liver disease. In a multiple linear regression model, only sum BA was kept as explanatory variable for KIM-1, while bilirubin was excluded. Furthermore, analysis of ASBT expression in kidney biopsies from CN patients showed preserved expression which further highlights the translational relevance of the finding in mice. In conclusion, BA enrichment in TEC is an early key event in CN pathogenesis. Inhibiting renal

ASBT and consequently BA uptake into TEC prevents CN and systemically decreases BA concentrations.

L1.02 Pharmaceutical inhibition of JNK/c-Jun-signalling in S. mansoni-infected mice aggravated liver damage

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DOI 10.1055/s-0043-1777460

Introduction Schistosomiasis, a neglected tropical disease, affects >240,000,000 people worldwide(1,2). Paired adult worms produce about 300 eggs daily, eventually provoking granulomatous liver fibrosis. c-Jun, a transcription factor that is involved in hepatocellular regeneration, proliferation, and apoptosis, is permanently induced by S. mansoni infection(3). We aimed to characterize the effects of pharmaceutical inhibition of c-Jun in S. mansoni-infected mice.

Methods Twelve eight-week-old C57BL/6-mice were infected with 100 cercariae (♂+♀) and nine non-infected littermates served as controls. Six weeks post-infection, mice were either supplied with JNK-inhibitor SP600125(4) (n=6; SP/Sm) or 0.9 % NaCl (n=6; Sm). Nine weeks post-infection, hepatic damage and biomolecular alterations were examined by western blotting, RT-qPCR, immunostaining, and functional tests. Group-differences ($p<0.05$) were statistically validated via one-way-ANOVA or t-test (SPSS29.0.0.0).

Results Serum-ALT levels significantly increased in infected mice (130U/L) compared to non-infected controls (30U/L) and enhanced following JNK-inhibition (165U/L). Concurrently, de-Ritis-ratio decreased under JNK-inhibition compared to S. mansoni-infection (Sm 1.62, SP/Sm 1.33). Similarly, CXCL2 and TH2-specific cytokines (IL-4, IL13) were prominently induced after JNK-inhibition (CXCL2 Sm 6.9-fold, SP/Sm 12-fold; IL4 Sm 205-fold, SP/Sm 334-fold; IL13 Sm 189-fold, SP/Sm 270-fold). Increased STAT3 phosphorylation (Sm 11.2-fold, SP/Sm 15.6-fold) was observed, which proposes the activation of a possible alternative signalling pathway. Serum triglyceride levels and hepatic expression of lipid metabolism-related genes were reduced in S. mansoni-infected animals with stronger tendencies to decrease after JNK-inhibition.

Conclusion The current results suggest that the inhibition of JNK/c-Jun-signalling in S. mansoni-infected mice alters immune responses and metabolism, leading to more severe hepatocellular damage.

L1.03 ECM1-Mediated Inhibition of Protease-Induced LTGF-β1 Activation in Chronic Liver Disease: Implications for Therapeutic Strategies

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Chronic Liver Disease (CLD) is characterized by progressive liver fibrosis, where the dysregulation of transforming growth factor-β 1 (TGF-β1) activation plays a pivotal role. Our study investigates the mechanism of extracellular matrix protein 1 (ECM1) in modulating LTGF-β1 bioactivity and its impact on CLD progression.

Using DESeq analysis, we observed increased expression of thrombospondins (TSPs), ADAMTS proteases, and matrix metalloproteinases (MMPs) in ECM1-KO mice with severe liver damage, alongside fibrotic gene upregulation. In immortalized LX-2 HSCs and primary human HSCs (phHSCs), recombinant ECM1 treatment or ECM1 overexpression prevented TSP-1-, ADAMTS1-, and MMP-2/9-mediated LTGF-β1 activation. Co-immunoprecipitation and in vitro interaction assays demonstrated that ECM1 inhibits LTGF-β1 activation through interaction with TSP-1 and ADAMTS1 respective sequences KRFK or KTFR, while also blunting MMP-2/9 proteolytic activity. In mice, AAV8-mediated ECM1 overexpression attenuated LTGF-β1 activation and liver fibrosis, while KTFR injections reversed ECM1-KO-induced liver injury. Clinical analysis revealed a correlation between decreasing ECM1 expression and increasing TSP-1, ADAMTS1, MMP-2, MMP-9, and LTGF-β1 activation in CLD patients. A mathematical model further validated the impact of ECM1 restoration on reducing LTGF-β1 activation, HSC activation, and fibrotic collagen deposition.

Our findings underscore the hepatoprotective effect of ECM1, which inhibits multiple protease-mediated LTGF-β1 activation pathways. During CLD progression, decreased ECM1 levels allow excessive protease-mediated LTGF-β1 activation, leading to fibrogenic signaling and worsening hepatic fibrosis. These insights suggest that restoring ECM1 or analogous peptides to the liver could serve as a novel, safer therapy targeting TGF-β1 in CLD.

Lecture Session II CLINICAL HEPATOLOGY, SURGERY, LTX 26/01/2024, 15.15pm–16.00pm, Lecture Hall

L2.01 Rapid liver regeneration following PVE/HVE improves overall survival compared to PVE alone – A midterm analysis of the multicenter DRAGON 0 cohort

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DOI 10.1055/s-0043-1777462

Background Hypertrophy-inducing procedures, such as portal vein embolization (PVE), improve future liver remnant (FLR) volume and function and help overcome limitations to resection. Mainly due to tumor progression while awaiting sufficient liver growth, 30-40 % fail to achieve surgery after PVE. In the DRAGON 0 study, simultaneous portal and hepatic vein embolization (PVE/HVE) has shown to increase FLR-hypertrophy, kinetic growth rate and resectability substantially compared to PVE alone. The purpose of this study is to compare the 3-year overall survival after PVE/HVE versus PVE in patients undergoing liver resection for primary and secondary cancers of the liver.

Methods In this multicenter retrospective study, all DRAGON 0 centers provided 3-year follow-up data of all DRAGON 0-included PVE/HVE and PVE cases between 2016 and 2019. Kaplan-Meier analysis was performed to assess 3-year overall and recurrence-free survival. Factors affecting survival were analysed using uni- and multivariable cox regression analysis.

Results In total, 199 patients from 7 centers were included, of which 39 underwent PVE/HVE and 160 PVE alone. Groups differed in median age ($p=0.008$). As reported previously, PVE/HVE resulted in a significantly higher resection rate compared to PVE alone (92 % vs. 68 %; $p=0.007$). A significant higher 3-year overall survival was observed in the PVE/HVE group (PVE/HVE: not reached after 36 months vs. PVE: 20 months, $p=0.004$). PVE/HVE was an independent

predictor of survival in uni- and multivariable analyses (hazard ratio: 0.46 (confidence interval: 0.27-0.76), p = 0.003).

Conclusion Overall survival after PVE/HVE is better than after PVE alone in patients with primary and secondary liver tumors

L2.02 Monitoring thiopurine metabolites and adding allopurinol optimizes thiopurine profiles and treatment response in autoimmune hepatitis

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Background In this multicenter study we aimed to establish the utility of defining a therapeutic range for thiopurine metabolites in autoimmune hepatitis (AIH), explore the impact of modifying thiopurine dosage, and assess the effect of adding allopurinol in non-responders without complete biochemical remission (BR).

Methods Metabolite concentrations were tested in 328 patients from four European centers (Hamburg, Nijmegen, Debrecen, Birmingham) at a total of 633 time points. Allopurinol was added in 34 non-responders. Patients were stratified based on single time point (STP) or multiple time point (MTP) testing.

Results Results from the STP group revealed no significant difference in median 6-TGN concentrations between treatment responders (BR: 202pmol/0.2ml, IQR 115-313) and non-responders (No BR: 204pmol/0.2ml, IQR 146-339, p = 0.27). In the MTP group, higher 6-TGN levels (BR: 245pmol/0.2ml, IQR 194-310), with a determined optimal cutoff of 250pmol/0.2ml, were associated with long-term remission. Increasing the average thiopurine dose by 33 % led to a modest increase in 6-TGN levels (162 vs. 194pmol/0.2ml, p = 0.0049), while 6-MMP levels markedly increased (553 vs. 1008pmol/0.2ml, p = 0.0001). This dose adjustment reduced ALT levels (63 vs. 45U/l, p = 0.001). In a difficult-to-treat cohort, adding 100mg allopurinol to low-dose thiopurines raised 6-TGN (176 to 321pmol/0.2ml, p = 0.0001) and lowered 6-MMP (2727 to 174pmol/0.2ml, "p < 0.0001"), accompanied by improved serum transaminases (75 vs. 33U/l, p = 0.0001).

Conclusion Maintaining remission in AIH often requires 6-TGN concentrations of at least 200-250pmol/0.2ml, which can be challenging to achieve due to preferential 6-MMP formation. Co-administering allopurinol effectively optimizes metabolite profiles and enhances biochemical response.

L2.03 Bedeutung von intrapulmonalen Shunts und dem hepatopulmonalen Syndrom für den klinischen Verlauf von Patienten nach TIPS-Anlage

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DOI 10.1055/s-0043-1777464

Zielsetzung Eine differenzierte Patientenselektion ist essenziell, um bestmögliche Resultate durch die Implantation eines transjugulären intrahepatischen portosystemischen Shunts (TIPS) zu erreichen. Die Relevanz von intrapulmonalen Shunts (IPVD) und dem hepatopulmonalen Syndrom (HPS) ist in diesem Kontext ungeklärt. Daher wurde in dieser Studie die Bedeutung von IPVD und HPS für den klinischen Verlauf von Patienten nach TIPS-Implantation untersucht.

Methodik Kontrastmittel gestützte Echokardiographie und Blutgas-Analysen (BGA) wurden angewendet, um das Vorliegen von IPVD und HPS zu prüfen. Multivariable competing risk Analysen, adjustiert nach dem FIPS-Score und therapierefraktärem Aszites als TIPS-Indikation, wurden durchgeführt, um die Endpunkte Lebertransplantations(LTx)-freies Überleben, sowie die Inzidenz von hepatischer und kardialer Dekompensationen im 1-Jahres Follow-Up zu analysieren.

Ergebnisse In die finale Analyse wurden 265 Patienten inkludiert. 51 % (n = 136) hatten IPVD und 27 % (n = 71) erfüllten die HPS- Kriterien. Patienten mit IPVD hatten einen niedrigeren FIPS-Score (-0,3 ± 0,8 vs. -0,1 ± 0,7, p = 0.047) und häufiger Varizenblutung als TIPS-Indikation (29 % vs. 16 %, p = 0.010). Patienten mit IPVD hatten signifikant häufiger hepatische Dekompensationen (HR: 1.84 (1.26-2.70); p = 0.002) und kardiale Dekompensationen (HR: 1.76 (1.01-3.05); p = 0.046). Hingegen war das Vorliegen von IPVD nicht mit einem niedrigeren LTx-freiem Überleben nach TIPS-Anlage assoziiert (HR: 1.081 (0.63-1.86); p = 0.780). Das Vorhandensein eines HPS war mit einem numerisch erhöhten Risiko für hepatische Dekompensationen (HR: 1.46 (0.93-2.28); p = 0.097) und kardiale Dekompensationen (HR: 1.71 (0.93-3.12); p = 0.082) assoziiert. Im Gegensatz dazu zeigte sich bei Patienten mit HPS kein Unterschied im LTx-freiem Überleben (HR: 1.05 (0.58-1.92); p = 0.870).

Schlussfolgerung Die Bestimmung vom IPVD-Status vor TIPS-Anlage könnte helfen, Patienten mit einem höheren Risiko für kardiale und hepatische Dekompensationen zu identifizieren.

Lecture Session III METABOLISM (INCL. MASLD) 26/01/2024, 17.50pm–18.35pm, Lecture Hall

L3.01 Patients with primary sclerosing cholangitis exhibit a specific metabolomic and lipidomic profile

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DOI 10.1055/s-0043-1777465

Background For disease monitoring in patients with inflammatory bowel disease (IBD) and primary sclerosing cholangitis (PSC), accurate biomarkers are a prerequisite for individual disease characterization and personalized therapy. This study introduces metabolic profiling in serum samples of patients with PSC as a promising approach for biomarker determination. The study aimed to characterize metabolomic and lipidomic profiles in serum samples of patients with PSC, IBD, and other extraintestinal manifestations (EIM), as well as healthy controls (HC).

Method Nuclear magnetic resonance (NMR) spectroscopy analysis was performed in serum samples of 33 patients with PSC compared with 40 age-, sex-, and body mass index (BMI)-adjusted HC and 64 patients with IBD and other EIM. Classification of patients was performed using partial least squares discriminant analysis (PLS-DA) and univariate and multivariate analysis methods.

Results Multivariate analysis revealed significant differences in metabolomic and lipidomic profiles in patients with PSC, IBD with other EIM, and HC. Lipidome profiles of patients with PSC showed increased levels of IDL particles, IDL

ApoB, and free VLDL-4 cholesterol compared with IBD/EIM and HC. In addition, lipids bound to VLDL-4, including cholesterol, triglycerides, and phospholipids, were increased in PSC compared with HC. Furthermore, increased pyruvic acid concentrations were found in both PSC and IBD/EIM patients compared to HC.
Conclusion Here, we describe for the first time specific metabolic fingerprints in serum samples of patients with PSC. These findings contribute to the differentiation of PSC patients from HC and patients with IBD and EIM, improving personalized therapeutic approaches and enhancing diagnostic confidence.

L3.02 Caspase 8 deletion reverses MASH progression following hepatocyte-specific JNK deletion

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DOI 10.1055/s-0043-1777466
Background Metabolic dysfunction-associated steatotic liver disease (MASLD) is a prevalent chronic liver disorder in the Western world. However, the mechanisms driving its progression to cirrhosis and hepatocellular carcinoma (HCC) remain unclear. c-Jun N-terminal kinases (JNKs) are known to play a significant role in liver health and disease. This study investigates the relevance of hepatocyte-specific JNK activation during experimental MASH initiation and progression.
Methods NASH was induced by a Western-style diet (WSD) in wild-type (WT) and hepatocyte-specific Jnk1 and Jnk2 deletion (JNK1/2Δhepa) mice. Caspase8/JNK1/2Δhepa mice were generated for further analysis.
Results JNK1/2Δhepa mice after WSD showed significantly increased transaminases, fibrosis, and inflammation compared to WT mice, evidenced by immune cell infiltration and elevated liver cytokines/chemokines. Gene pathway analysis revealed upregulation of apoptotic pathways, confirmed by TUNEL and Cleaved Caspase 3 staining. Adding caspase 8 deletion to JNK1/2Δhepa mice on WSD reduced transaminases, inflammation, fibrosis, and cell death. Intriguingly, pathway analysis demonstrated that most genes upregulated in JNK1/2Δhepa mice were reversed following additional caspase 8 knockdown. Deconvulation of RNA bulk data, based on unpublished liver single-cell datasets, revealed distinct increased immune cell populations in JNK1/2Δhepa mice, especially infiltrating macrophages and dendritic cells, which were reduced in Casp8/JNK1/2 Δhepa mice.
Conclusion Our findings suggest that Jnk1 and Jnk2 in hepatocytes play an essential role in modulating the oxidative stress response, which directs MASH initiation and progression via apoptotic cell death. Importantly, hepatocyte-specific caspase 8 is essential to modulate this response, suggesting caspase 8 as a promising therapeutic target for MASH.

L3.03 PNPLA3 fatty liver risk allele was fixed in Neanderthals and segregates neutrally in humans

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DOI 10.1055/s-0043-1777467
Fat deposition in human liver is modulated by environmental and genetic factors including the PNPLA3 p.I148M variant. When and how this variant evolved in humans has not been studied to date. Here we re-analyse ancient DNA to track the history of this allele throughout human history.
Published 6444 ancient and 3943 present-day genomes were used for analysis after extracting genotype calls for PNPLA3 p.I148M. To quantify changes through time and space, logistic and linear regression analyses were performed. To compare these changes with expected changes due to neutral factors such as genetic drift, we compiled a reference dataset of 1000 randomly selected SNPs for genome wide analysis.
The ancestral (reference) allele is fixed among all great apes. In contrast, on the human lineage, all available Neanderthal (n = 21) and Denisovan individuals (n = 2) either exclusively carried the risk allele or had missing data (n = 7) suggesting fixation of the allele in the ancestor of all archaic humans. Allele frequencies in modern human populations range from very low in Africa to > 50 % in Mesoamerica. Over the last 15,000 years, distributions of ancestral and derived alleles roughly match the present day distribution, including a high frequency in the Americas even in the earliest samples from 10,000BP. Logistic regression analyses did not yield signals of natural selection.
Our observation might underscore the advantage of fat storage in cold climate, particularly for Neanderthal under ice age conditions. The negative genome-wide analysis without signals of natural selection during modern human history does not support the thrifty gene hypothesis.

Lecture Session IV TUMORS 27/01/2024, 09.10am–09.55am, Lecture Hall

L4.01 Tumour-specific activation of a tumour-blood transport that may improve the diagnostic accuracy of blood tumour markers

Autorinnen/Autoren Christian Schmithals[1], Bianca Kakoschky[1], Dominic Denk[1], Maike von Harten, Klug Jan Hendrik[1], Edith Hintermann[2], Anne Dropmann[3], Eman Hamza[1], Anne Jacomin[1], Jens Uwe Marquardt[4], Stefan Zeuzem[2], Peter Schirmacher[6], Urs Christen[2], Thomas Vogl[2], Oliver Waidmann[2], Steven Dooley[3], Fabian Finkelmeier[2], Albrecht Piiper[1]
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DOI 10.1055/s-0043-1777468
The accuracy of blood-based early tumor recognition is compromised by signal production at non-tumoral sites, low amount of signal produced by small tumours, and variable tumour production. Here we examined, whether tumour-specific enhancement of vascular permeability by the particular tumour homing peptide, iRGD, which carries dual function of binding to integrin receptors overexpressed in tumor vasculature and is known to promote extravasation via neuropilin-1 receptor upon site-specific cleavage, might be useful to improve blood-based tumour detection by inducing a yet unrecognized vice versa tumour-to-blood transport. To detect an iRGD-induced tumour-to-blood transport, we examined the effect of intravenously injected iRGD on blood levels of α-fetoprotein (AFP) and autotaxin in several mouse models of hepatocellular carcinoma (HCC) or in mice with chronic liver injury without HCC, and on prostate-specific antigen (PSA) levels in mice with prostate cancer. We found that intravenously injected iRGD rapidly and robustly elevated the blood levels of AFP in several mouse models of HCC, but not in mice with chronic liver injury. The effect was primarily seen in mice with small tumours and normal basal blood AFP levels, was attenuated by an anti-neuropilin-1 antibody, and depended on the concentration gradient between tumour and blood. iRGD treatment was also able to increase blood levels of autotaxin in HCC mice, and of PSA in mice with prostate cancer. We conclude that iRGD induces a tumour-to-blood transport in a tumour-specific fashion that has potential of improving early stage cancer diagnosis.

L4.02 SBNO1 is an essential epigenetic driver with common and distinct roles in HCC and CCA

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DOI 10.1055/s-0043-1777469

Background Aberrant Notch signaling is a potential driver of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA). Genetic studies in Drosophila showed that the strawberry notch (sno) knockout mimics loss of Notch and thus, we elucidated the role of the mammalian Strawberry Notch 1 (SBNO1) in HCC and CCA development.

Methods HCC and CCA gene expression and proteomics datasets were analyzed for SBNO1 expression. Tissue microarrays were immunohistochemically evaluated for SBNO1 protein expression. SBNO1 was downregulated using siRNA or sgRNA in cell lines followed by cell viability, colony formation and migration assays and gene expression analysis. To identify SBNO1 protein interaction partners, BioID was performed. Syngeneic mouse models using Hep55.1C cells and hydrodynamic tail vein injection (HDTV) were applied.

Results SBNO1 protein but not mRNA was significantly increased in HCC and CCA and SBNO1 protein localized to the nucleus suggesting a role in gene regulation. SBNO1-inhibition reduced cell viability, colony formation and migration. SBNO1-knockdown induced distinct expression patterns in HCC and CCA cell lines, however, BioID revealed that SBNO1 similarly modulates gene regulation in HCC and CCA by binding to general transcription factors TAF4 and TAF3. Sbno1 knockout in Hep55.1C reduced tumor growth in vivo and inhibited liver tumor development in three different models of HCC and CCA using HDTV. Furthermore, Sbno1-deletion reduced biliary differentiation and angiogenesis in the tumor margin indicating that Sbno1 is necessary for Notch-driven CCA formation.

Conclusions We identified SBNO1 as a new epigenetic driver required for HCC and iCCA tumor cell proliferation in vitro and in vivo.

L4.03 Machine learning for liver cancer risk stratification on population-level data

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DOI 10.1055/s-0043-1777470

Hepatocellular carcinoma (HCC) is a highly fatal malignancy whose incidence is increasing due to the global obesity epidemic. Early diagnosis is crucial for curative therapy, but many patients are diagnosed at advanced stages with poor prognosis. To improve risk stratification and integrate the multi-dimensional well characterized risk constellations (chronic liver disease, serum indicators, lifestyle, hereditary risk), it is essential to standardize risk stratification and harvest the potential of big data. Population-based databases, such as the UK-Biobank (UKB), are an invaluable tool for this task. The UKB is a population-wide database with electronic health records, death registers, lifestyle, physical and biological measures as well as genomics (n = 500k each) and metabolomics data (n = 250k). Here, we train a random forest machine learning (ML) classifier on multimodal data of all included patients to predict HCC occurrence (n = 470). Training and testing a five-fold cross validation random forest model on 18 UKB centers inside England reveals high accuracy with a mean AUROC of 0.87. To emphasize the relevance of this approach, preliminary results reveal an astounding distribution of feature relevance, with very high relevance of blood and metabolomic parameters. This is followed by lifestyle and EHR parameters, while genetic information only mildly improves the predictions.
In conclusion, leveraging the comprehensive data from the UK Biobank via a random forest ML classifier underscores the importance of blood and metabolomic parameters in HCC risk prediction, while also highlighting the nuanced contributions of lifestyle, EHR, and genetic factors in enhancing diagnostic accuracy.

Lecture Session V VIRAL HEPATITIS AND IMMUNOLOGY 27/01/2024, 11.40am–12.25pm, Lecture Hall

L5.01 Sustained macrophage alterations during chronic injury regression beget increased susceptibility to infections

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The liver serves as the body's central microbial filter preventing spread of blood-borne pathogens. To fulfil this critical task, the liver relies on a hub of immune surveillance, the liver sinusoids which contain the most abundant population of tissue resident macrophages, the Kupffer cells (KCs). In homeostasis, KCs filter > 90 % of disseminated bacteria within minutes from the blood. In contrast, during chronic liver injury and fibrosis KCs dramatically change their phenotype, lose their identity and their function. Furthermore, monocytes invade the liver with an individual functional profile and form KC-like syncytia that compensate for loss of KC function on the level of sinusoids. However, it is unclear how the liver macrophage compartment responds to injury regression which is seen in patients with chronic liver diseases. Using a mouse model of chronic toxicity and regression of hepatic injury, numerous monocyte and macrophage linage tracing tools, intravital microscopy and multiplex flow cytometry we investigated the liver macrophage compartment during injury regression. Liver architecture and liver damage completely normalized in regression. Surprisingly, we found sustained alterations macrophage compartment that included differences in KC identity molecules such as CRIg and TIM-4. Functionally, bacterial capture was reduced in regression compared to control mice. Flow cytometric profiling revealed an emerging population of monocyte-derived KCs. Furthermore, we found a novel macrophage subset expressing the markers MerTK, CD68, CD86 and PD-L1. Our results demonstrate sustained alteration of the liver macrophage compartment during injury regression, favoring injury repair over antimicrobial responses thus leaving the host vulnerable to infections.

L5.02 Molecular pathways defining human CD8 T cell auto-aggression in autoimmune liver diseases

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Introduction Immunopathology in autoimmune liver diseases (ALD) such as autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC) or non-alcoholic steatohepatitis (NASH) is considered to be caused by T cells but the identity of auto-antigens activating auto-reactive T cells remains unknown. Here, we report the identification of auto-aggressive T cells (aaT cells) in liver tissues of patients with ALD that developed in response to IL-15-mediated metabolic reprogramming, and efficiently killed hepatocytes in the absence of MHC-restricted antigen recognition.

Methods Single-cell RNA-seq of CD8 T cells (ex vivo/in vitro), metabolic flux analysis, flow cytometry and cytotoxicity assays were performed to study phenotype and immunity of human aaCD8 T cells.

Results scRNA-seq of IL-15 activated human CD8 T cells in vitro identified CD8 T cells with an auto-aggression signature that was characterized by high levels of HLA type II molecules and granzymes. Using bioinformatic approaches, we found CD8 T cells with an auto-aggression gene signature to be enriched in tissues of patients with ALD. Mechanistically, IL-15 induced a SGK1-dependent hyper-charged metabolic program in CD8 T cells that provided high amounts of ATP which was required for the acidification of high numbers of cytotoxic vesicles in aaCD8 T cells. Consequently, aaCD8 T cells that were activated by extracellular histones eliminated target cells through a granzyme-dependent mechanism with similar rapid killing dynamics like NK cells.

Conclusion We defined molecular pathways of MHC-unrestricted CD8 T-cell auto-aggression in tissues of patients with ALD that could open new avenues for the treatment of ALD and other autoimmune diseases.

L5.03 Spatial immunological analysis of novel immune-mediated liver diseases during the pandemic

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Background During the pandemic, a rise in cases of severe hepatitis in children of unknown origin has been noted. Several hypotheses are discussed, such a connection to HAdV infections in combination with a second viral infection or exuberant post-COVID immunity.

Methods Immune and non-immune cell phenotyping of 12 formalin-embedded liver tissue sections obtained from pediatric patients presenting with acute hepatitis (≤ 16 years old, ALT (U/L) > 500) was performed using imaging mass cytometry (IMC). The 40 + marker panel focused on liver parenchyma and immune markers related to proliferation, activation, and T cell exhaustion. SARS-CoV detection was performed using PCR, serology, immunofluorescence, and immunohistochemistry.

Results 8/12 patients displayed pronounced periportal immune cell infiltrations accompanied by elevated liver enzyme levels. In contrast, 4/12 patients displayed an immune cell landscape compatible to control liver biopsies. Notably, CD8 T cells and myeloid cells were found to be the predominant immune cell populations. Additionally, granulocytes and CD8 T cells interacted with biliary tracts and periportal endothelial cells. 2 patients tested positive for HAdV + . In all pediatric patients, SARS-CoV was detected to various degrees in collagen-rich areas surrounded by immune cells.

Conclusions Our findings highlight the presence of SARS-CoV antigen in the liver tissue of pediatric patients with acute hepatitis of unknown etiology that occurs after resolution of clinically manifested Covid19, suggesting a potential association between SARS-CoV infection, immune cell infiltrations and the observed hepatic manifestations.

Poster Visit Session I BASIC HEPATOLOGY (FIBROGENESIS, NPC, TRANSPORT) 26/01/2024, 12.30pm–13.00pm

P1.01 IL-6 Trans-signaling Controls Liver Regeneration After Partial Hepatectomy

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The process of liver regeneration following partial hepatectomy is a remarkable physiological response that involves intricate molecular mechanisms. Among the key players in this regenerative process is interleukin-6 (IL-6), a multifunctional cytokine known for its diverse roles in immune and inflammatory responses. IL-6 signals through two distinct pathways: the classical pathway, involving the membrane-bound IL-6 receptor (IL-6R), and the trans-signaling pathway, which relies on a soluble form of IL-6R. However, the specific contributions of these pathways to liver regeneration have remained elusive.

This study aimed to unravel the specific roles of IL-6 trans-signaling in the context of liver regeneration after partial hepatectomy. Using a novel transgenic mouse model with selective IL-6 trans-signaling activation, we systematically investigated the impact of this pathway on hepatocyte proliferation and tissue repair. Our findings unequivocally demonstrate that IL-6 trans-signaling is a critical regulator of liver regeneration. In terms of mechanisms, we demonstrated that IL-6 trans-signaling triggers the synthesis of hepatocyte growth factor from hepatic stellate cells, culminating in the stimulation of hepatocyte proliferation and facilitating tissue repair.

These findings have significant implications for therapeutic strategies aimed at promoting liver regeneration in the context of liver injury and disease. By elucidating the precise contributions of IL-6 trans-signaling to liver regeneration, this study not only enhances our understanding of fundamental regenerative processes but also highlights potential targets for interventions to enhance tissue repair and recovery. Our work underscores the importance of dissecting specific signaling pathways in deciphering complex biological phenomena and advancing translational research for clinical benefit.

P1.02 Improved culture of primary hepatocytes in a platform suitable for the establishment of an advanced in vitro liver model

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The successful in vitro culture of functional primary hepatocytes is essential for drug toxicity tests and the modelling of liver diseases. This remains difficult due to the rapid dedifferentiation of primary hepatocytes during culture. There are many approaches to inhibit hepatocyte dedifferentiation, but these oftentimes lack the physiological arrangement of hepatocytes or sufficient hepatocyte function. Here, a three-dimensional (3D) matrix is used to inhibit hepatocyte dedifferentiation during culture in a platform suitable for the establishment of an advanced in vitro liver model.

For this, freshly isolated primary murine hepatocytes were cultured within gelatin-methacryloyl (GelMA) hydrogels.

The 3D GelMA environment significantly improved hepatocyte-like function of the encapsulated cells compared to the standard 2D culture. Importantly, hepatocyte viability was massively enhanced when hydrogels were cultured in

gas permeable well plates. Furthermore, cell density and cell-cell contact were improved by culturing hepatocytes in GelMA with an additional melt electrowritten fiber scaffold. However, scaffold addition enhanced the expression of the dedifferentiation marker vimentin, while maintaining hepatocyte-specific gene expression compared to GelMA [1].

In conclusion, the 3D matrix and enhanced oxygen supply significantly improved hepatocyte viability and hepatocyte-like function. This underlines the demand for a perfused hepatocyte culture system to further increase oxygen and nutrient supply, which is aimed to be achieved by using perfusion chambers with endothelialized microvascular networks1. Furthermore, the hydrogel composition and stiffness should be adapted to achieve a more physiological morphology of the hepatocytes while reducing expression of the dedifferentiation marker.

Literatur

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P1.03 Multimodal and site-specific differentiation trajectories of cDC2 in liver damage and cholestasis

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Background Functional maturation of conventional dendritic cells (cDCs) is dependent from biliary epithelial cell (BEC)-derived signals that drastically change in liver cholestasis. BEC niche composition and portal inflammatory dynamics of cDC2 remain poorly understood. Here, dynamical changes of cDC2 subtypes, heterotypic communication patterns with BECs and the impact of cDC2 on site-specific T cell polarization are analysed.

Methods A hepatic 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet scRNA-seq atlas containing 5 timepoints and 73000 cells was generated. Transcription factor regulon activity was predicted using SCENIC. CITE-seq was used to distinguish different DC subtypes in liver and hepatic lymph nodes. Frequency changes were confirmed by FACS analysis, multicolor immunofluorescence and qRT-PCR.

Results DDC scRNA-seq data revealed a Sox9 + BEC-derived and Nfkb2-regulated, conserved gene expression programme that included DC-regulators such as Macrophage colony-stimulating factor 1 (Csf1), CC-chemokine ligand 2 (Ccl2) and tumor necrosis factor (Tnf). Prolonged cholestasis was associated with a cellular shift towards preDCs, while mature cDC2B accumulated in draining lymph nodes. VarID2 predicted independent cDC2 disease trajectories that included Interleukin 17 (Il17) inducing genes in tissue-resident cDC2 at onset and a perturbed cDC2 differentiation at prolonged cholestasis. Cholestasis was characterized by an unconventional T cell (UTC)-driven Il17-response and an influx of gamma delta T cells, which could be further subtyped based on Il17 expression and TCR repertoire.

Conclusion/Outlook Cholestatic liver disease is associated with a dynamic change in the cDC2 compartment which may affect UTC polarization. Currently, spatial transcriptomics and cell-specific depletion models are used to unwire the multicellular interplay at the biliary niche.

P1.04 The cell cycle protein Cyclin E1 mediates pro-inflammatory signals in a mouse model of acute hepatitis and in primary macrophages

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Cyclin E1 mediates important steps within the cell cycle. We previously identified unexpected essential functions of Cyclin E1 for liver fibrogenesis, inflammation and hepatocarcinogenesis. However, the effector cells of Cyclin E1 in the liver have not yet been fully identified. Here, we investigated the role of Cyclin E1 in immune cells during acute liver inflammation.

We used constitutive Cyclin E1 knockout (Ccne1-/-) mice and the Concanavalin A (ConA) model of immune-mediated hepatitis. Moreover, we investigated bone marrow-derived macrophages (BMDM) from Ccne1-/- and WT mice and performed knockdown experiments in a macrophage cell line using anti-Ccne1 siRNA encapsulated in lipid nanoparticles (LNP).

Ccne1-/- mice showed improved survival after ConA-treatment, which was associated with significantly reduced liver necrosis. Loss of Ccne1 was related to down-regulation of pro-inflammatory mediators such as interleukin-6 (IL6), tumor necrosis factor alpha (TNF), and CC-chemokine ligand 2 (CCL2). We tested the impact of Ccne1 deletion in pro-inflammatory polarized BMDMs. Ccne1-/- BMDMs also revealed reduced IL6 expression compared to WT controls. Importantly, Ccne1-/- BMDMs did not show any changes in cell cycle progression or in myeloid progenitor cell to macrophage differentiation. These findings were validated in an interventional approach. To this end pro-inflammatory polarized J774A.1 cells were treated with anti-Ccne1 siRNA-LNPs, which was sufficient to significantly reduce IL6 expression.

Altogether, Cyclin E1 mediates a novel, pro-inflammatory function in the liver, which is fully independent from its canonical role as a cell cycle mediator. Thus, inhibition of Cyclin E1 could be a promising approach for treatment of acute immune-mediated hepatitis.

P1.05 IL-6 receptor availability and IL-6/STAT3 signal transduction is regulated by ALR through altered sheddase ADAM17

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Interleukin 6 (IL-6), a key pleiotropic cytokine, plays a vital role for hepatic function and regeneration. Augmenter of Liver Regeneration (ALR) is a widely expressed co-mitogen with anti-apoptotic, anti-oxidative, and anti-inflammatory properties, often dysregulated in liver diseases. Application of ALR has been shown to dampen hepatic acute phase response (APR) triggered by IL-6. This study aimed to unveil the underlying molecular mechanism of how ALR affects IL-6 signaling pathway using in vitro experiments. Two hepatoma cell lines were treated with IL-6, in presence or absence of recombinant human ALR (rALR), and analyzed for expression as well as activation of IL-6 signaling cascade components by western blotting, qRT-PCR, protein activity assay and ELISA. Our findings demonstrate that rALR effectively reduces IL-6-induced STAT3 and JAK1/JAK2 phosphorylation, independent of IL-6 concentration. Expression and phosphorylation of SHP1, SHP2, SOCS1, SOCS3 and PIAS, all known negative regulators of STAT3 activity, remained unaffected by rALR. Furthermore, rALR treatment did not change IL-6 receptor-α (gp80) and IL-6R-β (gp130) mRNA expression. Western blot analysis of IL-6 receptor subunit expression was not conclusive due to low immune-signals. Nevertheless, we found elevated levels of soluble gp80 and gp130 receptor subunits in cell culture supernatants upon rALR treatment (with or without IL-6) by ELISA. Additionally, rALR enhances shedding activity of ADAM17 (TACE), which might be responsible for increased soluble and diminished membrane presence of IL-6 receptor. In conclusion, rALR attenuates hepatic IL-6 receptor availability and classical IL-6 signal transduction, but also may potentially affect IL-6 trans-signaling.

P1.06 Phosphorylation of α-and ß-catenin by ALR through EGF-Receptor dependent mechanisms may results in disruption of cell-cells contacts

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The liver is known for its remarkable regenerative capacity, primarily regulated by various factors, notably Augmenter of Liver Regeneration (ALR) and ß-catenin. ALR, has anti-oxidative, anti-apoptotic and anti-inflammatory properties and plays a pivotal role for hepatic functionality. ß-catenin is the key nuclear effector of canonical Wnt signaling and, in addition, serves as integral structural component of cadherin-based adherens junctions. ß-catenin may be released from membrane bound E-cadherin upon activation of EGF receptor (EGFR) followed by specific phosphorylation of α- and ß-catenin (catenin-complex). Since ALR can activate EGFR, this study aims to analyze the impact of recombinant ALR (rALR) on membrane-bound ß-catenin phosphorylation and its underlying signaling pathways. Hepatoma cell lines (HepG2, Huh7) were treated with rALR or EGF, and specific inhibitors of signal transduction pathways, followed by western blotting analysis. Application of rALR results in phosphorylation of ß-catenin at Y654 and S552, as well as α-catenin at S641. Phosphorylation of α-catenin at S641 is mediated by activation of ERK1/2 and ß-catenin at S552 by activation of PI3K/Akt signaling pathways – both downstream effectors of EGFR activation. Furthermore, rALR can activate src, a non-receptor tyrosin kinase, known to activate the EGFR and directly phosphorylate ß-catenin at Y654. Taken together, rALR activates EGFR tyrosine kinase by phosphorylation of EGFR at specific sites, which in turn activates src, MAPK and PI3/Akt pathways. Therefore, as reported for EGF earlier, rALR can lead to phosphorylation of α- and ß-catenin and their dissociation from E-cadherin-complex, loss of cell-cell contacts and nuclear translocation of ß-catenin.

P1.07 Myeloid-specific Pla2g6 deficiency aggravates sex-dependent hepatic and pancreatic apoptosis and fibrogenesis in aged mice

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Background Polymorphisms of PLA2G6 are associated with elevation of serum C-reactive protein, and PLA2G6 mutations lead to neurodegeneration and Parkinson's disease. Age-related abnormalities including hepatic fibrosis have been reported in global Pla2g6-null mice. We recently showed that myeloid-specific Pla2g6-deficient mice exhibited NASH susceptibility (BBA 2023). We therefore evaluated whether these mutants would exhibit inflammatory fibrogenesis during aging.

Methods Male and female Pla2g6-deficient and control (LysM-Cre and Pla2g6 Flox) mice at 24 months old were used. Plasma cytokine levels were determined by ELISA. The analyses of Western blot, histology, Sirius-red staining, and immunohistochemistry (IHC) of liver and pancreas were performed.

Results While male and female mutants showed a trend increase of spleen weights, only male mutants showed a decrease in pancreas weights. Mutants of both sexes displayed a significant increase in plasma LDH, TNFα, IL-6, IFN-γ, MIP-1α, IL-4, IL-5, IL-13 as well as IgM and IgG1. They concomitantly showed increased hepatic and pancreatic apoptosis as determined by Western blot and IHC of cleaved caspase3, respectively. Remarkably, female but not male mutants displayed an increase in Sirius-red(+) fibrosis and IHC CK19(+) cells in liver and pancreas indicating inflammatory ductal epithelial cell proliferation. Livers of female mutants also displayed an increase in IHC(+) of F4/80, CD3, CD45R, and eosinophil cationic protein suggesting exaggerated recruitment of immune cells.

Conclusion Associated with systemic inflammation, aged male and female mutants showed increased hepatic and pancreatic apoptosis, while exaggerated fibrogenesis was observed in female mutants only. Our results may be applicable to aged patients with PLA2G6 mutations.

P1.08 TLR9 deletion rescues cell death associated fibrosis- and hepatocarcinoma progression in models of liver injury

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Aim Toll-like receptor 9 (TLR9) serves as a sensor for unmethylated DNA derivatives from pathogens and damaged host cells. Hence, it plays an important role in immune response modulation. It has been reported that TLR9 expression correlates with a poor prognosis in patients with hepatocarcinoma (HCC), but also that TLR9 remodels the tumor microenvironment when stimulated. In this work, we aim to investigate the involvement of TLR9 in fibrosis- and hepatocarcinoma progression.

Methods We used a mouse line with constitutive deletion of Tlr9 (TLR9-/-), which was treated with DEN/CCl4 for 26 weeks. Additionally, hepatic NEMO knockout (NEMO(Delta hepa)) and a double knockout (NEMO(Delta hepa)TLR9-/-) were generated and used as a confirmatory model.

Results TLR9 deletion resulted in an overall lower HCC tumor burden and less hepatic fibrosis. We observed down-regulation of hepatic stellate cell activation and consequently decreased collagen production in both models. The lack of TLR9 is reflected in the inflammatory response by increased gene expression of pro-inflammatory cytokines such as like tumor necrosis factor alpha and interleukin-1 beta, together with a reduction of B cells and increased exhaustion of T cells in both models in the respective TLR9-/- group.

Conclusion Our data define TLR9 as a major contributor to fibrosis- and hepatocarcinoma initiation and progression in models of chronic liver injury. We show that TLR9 is primarily expressed in Kupffer cells and not hepatocytes, suggesting a key role of TLR9 in the intercellular communication during liver damage.

P1.09 The impact of activated MAIT-cells on cholestatic liver diseases

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Introduction Mucosa-associated invariant T (MAIT) cells are abundant in the human liver (1). Cholangiocytes and bile acids (BA) can activate MAIT-cells (2–4). Recently, depletion and alterations in MAIT-cells have been observed in various liver diseases, suggesting their impact on disease progression (5–7). The present study aims to characterize MAIT-cell-associated genes in cholestatic liver diseases, including the impact of BA receptor TGR5 on MAIT-cells.

Method Expression of MAIT-cell-associated genes in human and mouse livers were quantified by qPCR. To investigate the impact of TGR5 on MAIT-cells, TGR5 wildtype, knockout, and transgenic mice were analyzed. Co-culture experiments of isolated PBMCs, including cholangiocytes cell line H69 or human bile-derived organoids, were pre-stimulated for 24h. The frequency of recruited MAIT cells from PBMCs was measured by flow cytometry. Additionally, mRNA levels of bile acid receptors and MR1 were quantified after co-culture.

Results The mRNA expression of MAIT-cell-associated genes were upregulated in whole livers of PSC patients and Abcb4-/- mice. Conversely, the mRNA levels

were downregulated in whole livers of CCA and HCC patients, except for MR1. Co-culture experiments of isolated PBMCs with cholangiocytes led to an increased frequency of recruited MAIT-cells from PBMCs. Furthermore, H69 cells showed an increased expression of MR1 and BA receptors in co-culture with PBMCs.

Conclusions Alterations in the expression of MAIT-cell-associated genes have been observed in various liver diseases. These findings, coupled with the observation that cholangiocytes possess the ability to activate MAIT-cells, suggest the potential influence of MAIT-cells on the progression of liver diseases.

P1.10 Copper-induced H2O2 reduces NFκB and AP1 promoter activity in a Wilson's disease cell culture model

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Wilson's disease, an autosomal recessive disorder, is associated with ATP7B mutations. ATP7B encodes an ion pump that enables the transport of copper from hepatocytes into the bile. Inefficient ATP7B function leads to toxic copper overload with clinical effects on liver and brain function.

Our aim is to compare the transcriptional response to copper stimulation in wild type and ATP7B knockout hepatoma cells (HepG2). RNA sequencing data (GSE107323) were reanalysed using the Qlucore software and the expression of selected genes was validated in vitro (quantitative PCR). Gene expression was validated by quantitative PCR. NFκB and AP1 luciferase reporter, oxidative stress and functional assays were performed.

Multiple component analysis identified gene expression patterns in the ATP7B knockout (KO) cells that are associated with endoplasmic reticulum (ER) stress, autophagy, ion transport, cell intrinsic immunological signals (NFκB and AP1) and liver function. Quantitative PCR partially validated the expression of selected genes. CuCl2 treatment resulted in increased luciferase reporter activity in NFκB and AP1 promoter assays. CuCl2 treatment induced oxidative stress as determined by H2O2 measurement. Finally, H2O2 treatment directly reduced NFκB (ATP7B-KO HepG2) and AP1 (ATP7B-KO and wild type HepG2) promoter activity.

ATP7B knockout cells responds with increased autophagy to prevent copper-associated apoptosis. The present expression analysis indicated that next to metal ion transport and autophagy also genes involved in inflammation and liver function were effected. Finally, inflammatory signatures in ATP7B-KO HepG2 seem to be more effected by oxidative stress.

P1.11 Effects of rifaximin-α on liver phenotype in murine models of early stage alcohol-associated and metabolic-dysfunction associated steatotic liver disease

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Background Alcohol-associated (ArLD) and metabolic dysfuncion-associated fatty liver disease (MASLD) are currently the leading causes for chronic liver diseases in the Western world. Rifaximin-α is a non-absorbable antibiotic and in clinical use in advanced chronic liver disease for the treatment of hepatic encephalopathy. In this study, we aim to investigate the effects of rifaximin-α on the liver phenotype in different murine models of early stage ArLD and MASLD.

Methods 12-week-old, C57Bl/6 mice were treated for 7 weeks with either a Methionine-Choline deficient diet (MCD), Western diet (WD), carbon tetrachloride (CCl4) inhalations or standard chow (n = 20). Half of the mice were assigned to receive additionally ethanol. Every group was further divided whether to receive rifaximin-α (30 µg/mouse/day) or not. After sacrifice, livers were analyzed in order to determine fibrosis, inflammation, proliferation and steatosis through (immuno-)histochemistry, qPCR and photometric assays for hydroxyproline and triglycerides.

Results As expected, CCl4 treatment induced the strongest liver fibrosis. MCD treatment led to decreased body weight as well as liver steatosis and fibrosis. Additionally, ethanol increased fibrosis in MCD-treated animals, but decreased fibrosis in WD-fed mice. Rifaximin-α did not have any effect on liver fibrosis or steatosis, but a mild increase in proinflammatory transcripts were observed in MCD-based, WD + ethanol and ethanol-treated animals. In ethanol, MCD + ethanol and WD-treated mice, rifaximin-α treatment led to an increasing trend in hepatic proliferation.

Conclusion Liver fibrosis and steatosis of MCD- and WD-induced early murine MASLD and ArLD did not change during rifaximin-α treatment. However, mild changes in hepatic inflammation were observed.

P1.12 EGF/STAT1-maintained ECM1 expression in hepatic homeostasis is disrupted by IFNγ/NRF2 in chronic liver diseases

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In healthy liver, latent TGF-β is stored in the extracellular matrix and kept quiescent by ECM1. Upon damage, ECM1 production is downregulated in hepatocytes leading to L-TGF-β activation, thus inducing HSC activation and subsequent liver injuries. This study investigates the underlying regulatory mechanism of ECM1 expression in the liver under different pathophysiological conditions. Physiologically, ECM1 expression in hepatocytes is maintained by EGF/EGFR/STAT1 pathway. Blocking EGFR significantly inhibits ECM1 expression both in vitro and in vivo. Knockdown of Stat1 is sufficient to phenocopy EGFR inhibition, suggesting STAT1 as a downstream mediator of the EGFR signal to maintain ECM1 expression. STAT1 binding to the Ecm1 gene promoter was confirmed by ChIP assay. Upon liver inflammation and injuries, high levels of hepatic IFNγ intercepts EGF signaling through inhibiting EGFR expression, thereby interfering with homeostatic 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. Injection of IFNγ to mice also confirms in vitro findings. More importantly, patients with cirrhosis who have negative nuclear expression of NRF2 maintain relatively high level of ECM1, consistently exhibiting a better outcome.

Overall, ECM1 has the potential to be developed as an anti-fibrotic agent to improve the prognosis of chronic liver diseases.

P1.13 Kidney specific deficiency of the Ileal Bile Acid Transporter is hepatoprotective in a murine model of cholestatic liver disease

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Introduction The Ileal Bile Acid Transporter (Asbt, Slc10a2) is the major bile acid (BA) uptake mechanism in ileum and kidney tubules. Ileal ASBT inhibitors have been developed as a treatment of cholestasis. Cholestatic liver disease is often characterized by obstruction of bile flow into the duodenum, so that the benefit of ileal ASBT inhibition is limited here. The aim of our study was to determine if kidney specific ASBT inhibition is hepatoprotective in cholestatic liver injury and whether these mice are prone or protected against development of cholemic nephropathy.

Methods Eight-week-old male and female Asbt-fl/fl (control) and Asbt-fl/fl-Pax8 + (AsbtKKO) mice were fed chow or chow with 0.1 % DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine) for one week. Organs were collected for histology, immunohistochemistry (IHC) and RT-qPCR. Serum and urine were collected for biochemical analysis.

Results AsbtKKO mice fed with DDC had significantly increased urine BAs, decreased serum biomarkers of liver injury, and decreased signs of liver damage including reduced fibrosis and ductular reaction in Sirius Red staining and IHC respectively as compared to control under DDC diet. Urine creatinine excretion was decreased in all mice fed with DDC, but there were no signs of increased kidney injury in RT-qPCR and urine analysis in the AsbtKKO mice.

Conclusions Renal depletion of Ibat was hepatoprotective in a model of cholestatic liver damage in mice. Pharmacological inhibition of ASBT in kidney is a promising approach for patients with cholestasis and a dominant stenosis of the bile duct.

P1.14 Exploring the tissue specific roles of FXR within the gut-liver axis: Insights from a novel mouse model

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Background Farnesoid X receptor (FXR) agonist are already approved or are tested in patients with chronic liver diseases e.g. primary biliary cholangitis (PBC). FXR controls several variables within the gut-liver axis, however its distinct functions remain incompletely understood.

Methods In the present study, we have developed a novel mouse model using a tissue specific FXR knockouts for hepatocytes (FXRΔhepa), intestinal epithelial cells (FXRΔIEC) and a combination of both – the FXRΔhepaΔIEC animals.

Results FXRΔIEC mice showed only a mild cholestatic phenotype with liver function tests within the normal range, while FXRΔhepa mice exhibited a more severe phenotype with elevated transaminases, moderate cholestasis and hepatic inflammation. In contrast, 8-week-old combined FXRΔhepaΔIEC animals demonstrated exacerbated liver damage. Gene pathway analysis revealed an upregulation of mRNA levels of various genes involved in inflammatory pathways and immune response. FXRΔhepaΔIEC livers showed inflammation with increased infiltration of inflammatory cells, e. g. macrophages and neutrophilic cells. Bile acid analysis in serum, liver and feces showed a severe dysregulation. Fibrotic markers and Sirius Red staining of FXRΔhepaΔIEC livers were upregulated. In 52 weeks-old animals, dysplastic nodules were evident.

Conclusion Our data suggest that loss of either the intestinal or the hepatic FXR can lead to a minor phenotype, while its combined loss leads to severe cholestatic liver injury associated with increased liver fibrogenesis and malignant growth. Hence FXRΔhepaΔIEC animals are a novel model of progressive cholestatic liver injury and can contribute to a better understanding of the tissue-specific roles of FXR within the gut-liver axis.

P1.15 Identification of AP-1-inducing factors of Schistosoma mansoni-egg secreted proteins

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Questions Schistosomiasis is one of the most common parasitic infections of humans worldwide. The eggs of S. mansoni induce chronic granulomatous lesions in the liver and bowel of infected mammals. We have demonstrated that the protooncogene c-Jun is permanently induced in perigranolomatous hepatocytes and enterocytes by egg secreted proteins during S. mansoni infection. The current study aims to identify the AP-1-inducing S. mansoni-egg-secreted protein.

Methods We developed an AP-1 reporter gene assay by using the pGL4.44[luc2P]AP1 vector (Promega). Protein fractions of soluble egg antigens (SEA) were separated by molecular mass using liquid chromatography. The distribution of individual egg secreted factors were analyzed by western blotting in these fractions. SW620 cells were stimulated with the fractions and induction of the AP-1 specific promotor was analyzed by reporter gene assay.

Results While IPSE-containing SEA fractions induced a moderate activation of the AP-1 promotor, AP-1-promotor activity peaked with Omega-1 containing fractions. Enhanced phosphorylation of c-Jun in the cell lysates of IPSE and Omega-1-treated cells was demonstrated by western blotting.

Conclusion The results suggest that IPSE and Omega-1 are the most important proteins of S. mansoni egg antigens, which are involved in the activation of the AP-1 promotor. Further mechanistic studies using IPSE and Omega-1 enriched or depleted SEA may verify our current conclusions.

P1.16 Unravelling the molecular role of STBD1 in Hepatocytes: A Focus on Protein-Protein Interactions.

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Glycogen metabolism and autophagy are two processes that are influenced by the protein known as Starch Binding Domain 1 (STBD1). The C-terminal CBM20 domain is important for stability and protein interaction with GABARAPL1, PGYL, GYS1, HSPs, and AMPK. Our laboratory has shown a decrease in STBD1 expression in hepatocellular carcinoma (HCC), whereas other studies have shown a similar decrease in other types of cancers. Furthermore, STBD1 is localized to the endoplasmic reticulum (ER) and is potentially involved in cellular stress responses. This suggests that STBD1 may have different target proteins that have not yet been identified. Therefore, we study protein-protein interactions by exogenous expression of STBD1 with a V5-TurboID tag for proximity labeling with biotin and pull-down to identify the STBD1 interactome using mass spectrometry. To accomplish this, we cloned STBD1 into a V5-TurboID containing plasmid and a control plasmid lacking STBD1 and confirmed its expression in HepG2 cells. Next, we generated several stable monoclonal cells showing different expression levels, validated by qPCR and western blotting, to avoid experimental discrepancies. To date, each stable clone has shown consistent exogenous STBD1-V5-TurboID and V5-TurboID expression between cell passages. Second, STBD1-V5-TurboID localized to the nucleus, similar to calnexin, an endoplasmic reticulum marker, and TOM20, a mitochondrial marker, in our immunofluorescence studies. Thus, utilizing STBD1-V5-TurboID expressing stable cells for cellular and biochemical analyses enabled us to perform mass spectrometric studies. In summary, our investigations with STBD1-V5-TurboID expressing cells will identify the missing link between proteins that connect STBD1 to glycogen metabolism, autophagy, and HCC.

P1.17 Dissecting cell death and NF-kB in TAK1-deficient livers

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Question Hepatocellular carcinoma (HCC), the common end stage of chronic liver diseases, arises in the context of chronic hepatic inflammation. In chronic liver disease, hepatocyte cell death is a prominent feature driving inflam-mation and progression to hepatic fibrosis and HCC. The transcription factor NF-kB is one of the key regulators of inflammatory processes, but its function in hepatocarcinogenesis has remained controversial. Mice with conditional deletion of Tak1 (TGF-b-Activated-Kinase-1) in liver parenchymal cells (LPC; TAK1LPC-KO) display severe hepatic inflammation at young age characterized by inhibition of the NF-kB-signaling pathway and LPC apoptosis and necroptosis, proceeding over time to liver fibrosis and cancer, but also to severe lethal cholestasis. While either apoptosis or necroptosis has a fundamentally different impact on inflammation, cholestasis, and hepatocarcinogen-esis in TAK1LPC-KO mice, the function of NF-kB remains elusive.

Methods To examine the impact of reactivation of the NF-kB-signaling in TAK1LPC-KO mice, we crossed mice ex-pressing a Cre-dependent, dominant active form of the NF-kB-inducing kinase IKK2 (IKK2ca) with TAK1LPC-KO mice (TAK1LPC-KO/IKK2LPC-ca). The spontaneous phenotype of these mice was characterized and the molecular mecha-nisms underlying this phenotype were analysed by genetic, histological, and biochemical methods.

Results We demonstrated that reactivation of NF-kB abrogated hepatocarcinogenesis in TAK1LPC-KO mice due to the inhibition of LPC apoptosis, but exacerbated lethal cholestasis due to enhanced ductopenia.

Conclusion While reactivation of the NF-kB-signaling pathway in TAK1LPC-KO mice prevented hepatocarcinogen-esis, the active NF-kB-signaling exacerbated lethal cholestasis due to an unknown function of NF-kB in cholangio-cytes associated with the loss of bile ducts.

P1.18 Genetic variant in the hepatobiliary cholesterol transporter is associated with increased gallstone risk in the obese and with gallbladder cancer in general population

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Introduction Gallstone disease (GD) is prevalent but typically remains symptom-free. Nevertheless, complications such as choledocholithiasis or gallbladder cancer can arise from gallstones. Both genetic predisposition as well as exogenous factors are known to modulate the gallstone risk. Here we analyse the link between the gallstone-associated variant p.D19H in the hepatobiliary sterol transporter ABCG8 and the development of gallstone-related complications.

Methods Prospectively we recruited three cohorts of adult patients: 65 with gallbladder cancer, 170 obese individuals awaiting bariatric surgery, and 72 patients who required endoscopic retrograde cholangiopancreatography (ERCP) for recurring choledocholithiasis. Our control cohort included 172 adults without a history of gallstones. The ABCG8 p.D19H polymorphism was identified through TaqMan assays.

Results The minor allele frequency (MAF) of the examined variant was notably higher (P = 0.02) in cases with gallstones or gallbladder cancer (MAF = 8.4 %) compared to the control group (MAF = 4.0 %). The highest presence of the risk allele was observed in patients with gallbladder cancer (18.5 %) and obese patients with gallstone disease (17.5 %), succeeded by those with choledocholithiasis (13.9 %). Specifically, this variant was linked with an elevated risk of gallbladder cancer development (OR 2.54, 95 % CI 1.04-5.93, P = 0.02). It also amplified the risk of GD in obese individuals due for bariatric surgery (OR = 2.70, 95 %CI 1.05–6.49, P = 0.03), but it did not influence the risk for choledocholithiasis.

Conclusions The prevalent ABCG8 risk variant elevates the risk of gallbladder cancer and also intensifies the risk of gallstones in the obese. Individuals carrying the p.D19H variant might benefit from personalized preventative strategies.

P1.19 Inhibition of bacterial proteases prevents E-Cadherin cleavage in intestinal epithelial cells observed in spontaneous bacterial peritonitis

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Background Spontaneous bacterial peritonitis (SBP) is a serious bacterial infection in the peritoneal cavity, which typically arises in individuals with advanced liver disease, particularly cirrhosis. We previously identified a novel surface protease activity in SBP-inducing bacteria (Haderer et al., Gut, 2023), cleaving E-Cadherin of intestinal epithelial cells, and thereby disrupting intestinal epithelial barrier integrity. Inhibiting this protease activity could stabilize cell-cell contacts and potentially prevent SBP induction. Therefore, the identification of such inhibitors is of clinical relevance.

Method As an in-vitro model, HCT-116 cells were co-cultured with SBP patient-derived bacteria or a laboratory E.coli strain as internal reference. Since our studies demonstrated that the novel protease activity is sensitive to metalloprotease inhibitors (Haderer et al., Gut, 2023), various metalloprotease inhibitors were screened for their potential to prevent E-cadherin cleavage. Inhibitor effectiveness was evaluated using Western blot analyses and Azo-Casein activity assays.

Results The co-culture of HCT-116 cells with bacteria resulted in the rapid cleavage of E-Cadherin within 4 hours. This cleavage and disruption of cell-cell contacts were attributed to protease activity on the surface of the bacteria, as determined by an Azo-Casein protease activity assay. The activity of the bacterial protease was effectively inhibited by metalloprotease inhibitors (BB-94, BB-2516 and SB-3CT). Therefore, metalloprotease blockers have the potential to be a valuable tool for preventing disruption of the epithelial barrier.

Conclusion A newly identified bacterial protease activity cleaves E-Cadherin consequently contributing to destabilization of the epithelial barrier in SBP. Metalloprotease inhibitors efficiently prevent E-Cadherin cleavage and thus stabilize the epithelial integrity.

P1.20 Paraptosis – a new bacteria-induced cell death in intestinal epithelial cells and its role in the development of spontaneous bacterial peritonitis

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Background Spontaneous bacterial peritonitis (SBP) constitutes a severe complication of liver cirrhosis, carrying life-threatening implications. Pathophysiologically, SBP results from bacterial translocation from the gut into the perito-

neal cavity. However, mechanisms of bacterial interaction with intestinal epithelial cells are incompletely understood. Here, we link p53 family expression to paraptosis, a necroptotic cell death, induced by bacteria.

Method In vitro, we co-cultured HCT-116 cells with E.coli O6:Hnt and harvested the cells in 30-minute intervals for up to 4 hours. We investigated cell death mechanisms in HCT-116 wild-type and p53 family knockout (KO) cell lines (p53KO, p73KO, p53/p73 double KO) using specific inhibitors by flow cytometry. Protein expression of p53 and p73 was assessed through Western Blot, and cell morphology changes were examined via electron microscopy.

Results Bacterial co-culture-induced paraptosis in epithelial cell lines, marked by mitochondrial swelling, disruption of the ER and cytoplasmic vacuolization. Effective inhibition of cell death in both wild-type and knockout cell lines was achieved with the paraptosis inhibitor, actinomycin D. A CRISPR/Cas9-mediated knockout of p53 family members resulted in altered kinetics of cell death progression, with the absence of either p53 or p73 delaying paraptosis induction. This effect was most prominent in the p53/p73 double knockout cells.

Conclusion Bacterial co-culture induces paraptosis in intestinal epithelial cells, with the p53 family of transcription factors influencing cell death kinetics. The precise molecular understanding of the pathophysiology of SBP and the role of p53 in this condition will enable the development of new p53-dependent therapeutic approaches to prevent and treat SBP.

P1.21 Development of a 3D Intestinal Cell Culture System to Explore the Underlying Mechanisms of Spontaneous Bacterial Peritonitis (SBP)

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Background The application of 3D cell culture models, including spheroids, has emerged as a valuable tool for studying spontaneous bacterial peritonitis (SBP). We have previously identified a novel pathomechanisms in SBP (Haderer et al., Gut 2023), showing that intestinal bacteria specifically disrupt epithelial cell junctions in patients with liver cirrhosis. Here, we study the cleavage of essential cell-to-cell contact proteins in a 3D cell culture model.

Method Spheroids were generated using colon carcinoma cell lines Caco2 and HCT116, with Caco2 seeded at 30,000 cells/ml and HCT116 at 70,000 cells/ml. Spheroid formation was examined using fluorescence microscopy, revealing cell-cell contacts with vital roles for Occludin and E-Cadherin. Effects of a laboratory E. coli strain (O6:HNT) and SBP patient-derived E.coli (ONT:H41) on three-dimensional structure and integrity of cell junctions were examined by Western blot analysis and cell viability assays.

Results Caco2 and HCT116 cells formed distinct spheroids, confirmed by microscopy. The cells in the spheroids form cell-cell contacts. Co-culturing with a laboratory E. coli strain and patient-derived bacteria led to the degradation of cell-cell adhesion proteins: occludin diminished at 6 hours and E-cadherin that was cleaved after 9 hours. Additionally, bacterial co-culture induced cell death in spheroids.

Conclusion Understanding the pathophysiological changes in the intestinal epithelial surface of liver cirrhosis patients is crucial for developing SBP therapies. We show that bacteria-induced mechanisms, like cell junction disruption and the induction of cell death, can be modeled in spheroids. This establishes the foundation for creating biopsy-derived organoids, enabling highly personalized patient-specific models.

P1.22 The role of p53 and its isoforms in spontaneous bacterial peritonitis

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Introduction Spontaneous Bacterial Peritonitis (SBP) is a life-threatening complication of liver cirrhosis. In liver cirrhosis patients, a thinner mucus facilitates direct contact between bacteria and intestinal epithelial cells, resulting in the degradation of cell-cell contact proteins (Occludin and E-Cadherin). This leads to tissue damage and cellular stress (Haderer et al. Gut 2023). We investigate the association between tissue damage-induced cellular stress and p53 with a special focus on the induction of different p53 isoforms.

Methods To assess p53 isoform expression, we employed an exon-specific isoform expression reporter system (EXSISERS) integrated into exon 2, 4, and 7 of TP53 allowing us to distinguish three major isoform groups (anti-tumorigenic full-length p53 and Δ40p53 as well as oncogenic Δ133p53) in HCT116 colorectal cancer cells. Cells were co-cultured with the laboratory E.coli strain O6:Hnt, and the SBP patient-derived strains Ont:H7, and Ont:H41. p53 Isoform expression was evaluated.

Results Co-incubation with laboratory E.coli at an MOI of 5 resulted in the early upregulation of △40p53 expression within 15 minutes. Full-length p53 was induced after 4 hours. Simultaneously, we observed a gradual reduction in △133p53, known for its anti-apoptotic and pro-oncogenic properties. Thus, isoforms inducing cell death or cell cycle arrest were induced, and oncogenic isoforms were found to be downregulated.

Conclusion E.coli induce cellular stress by induction of tissue damage resulting in upregulation of pro-apoptotic p53 isoforms. These isoforms trigger cell cycle arrest and cell death. Cell cycle arrest helps cells adapt to bacterial stress, while increased cell death removes damaged cells.

P1.23 Testosterone as modulator of immune responses in female hepatic autoimmunity

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Background Women account for the vast majority of people who have autoimmune liver diseases but the mechanisms for this high female predominance are still unknown. Sex hormones are discussed as direct modulators of immune responses.

Aim & Methods To elucidate the influence of testosterone on CD4 + T cells, we combined ex vivo flow cytometry immunophenotyping, in vitro conversion assays and single cell CITE-sequencing on multiple cohorts: i) Females with PBC compared to age and sex matched controls, ii) transgender men receiving gender-affirming testosterone treatment and iii) a transman with an AIH/PSC variant syndrome receiving gender-affirming testosterone treatment.

Results Females with PBC presented with significantly decreased serum testosterone levels compared to age and sex matched healthy controls. Furthermore, we identified elevated frequencies of pro-inflammatory Th1 and Th17 CD4 + T cell subsets and increased in vitro Th1 conversion rates of naïve CD4 + T cells in females with PBC. To confirm in vivo effects of testosterone in humans, peripheral blood T cells from transmen receiving gender-affirming testosterone treatment were analysed before and during treatment. We observed a shift towards regulatory immune cell populations (Treg) and decreased proinflammatory T cell subsets including Th17 and Th1 cells frequencies upon six months treatment with testosterone. We furthermore analysed samples from a transman diagnosed with autoimmune liver disease and detected lower proinflammatory pathway and T cell activation marker expression upon treatment with testosterone.

Conclusion In summary, we here report a direct effect of testosterone on CD4 + T cells, shifting them towards anti-inflammatory phenotypes.

P1.24 Influence of CD44 on cell proliferation and fibrosis in Mdr2-/- mice

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Background Chronic cholestatic liver injury is linked to inflammation, the emergence of ductular reactions and fibrosis. While expression of CD44, a cellular adhesion molecule, is associated with liver fibrosis in different animal models, it remains unclear if CD44 has a functional role in the processes culminating in the development of hepatic fibrosis. In this study, we investigated the role of CD44 in chronic cholestatic liver injury in Mdr2-/- mice.

Methods Livers of Mdr2-/- (KO) and Mdr2-/-;Cd44-/- (DKO) mice were analyzed by histopathology and immunohistochemistry at different time points to assess liver damage, fibrosis and proliferation markers.

Results In three-months old DKO mice, proliferation in periportal areas was significantly lower than in age-matched Mdr2-/- controls. These findings correlated with reduced numbers of CK19-positive periportal cells in a higher level of fibrosis at 6 months in DKO mice in comparison to KO controls. Interestingly, CD44-deficient Mdr2-/- mice exhibited lower levels of active, nuclear YAP in periportal cells than controls, therefore indicating that CD44 might control proliferation in periportal cells via YAP.

Conclusions In chronic cholestatic liver injury in Mdr2-/- mice, deficiency for CD44 leads to reduced proliferation in periportal areas and decreased expansion of ductular reactions. These findings correlated with reduced YAP expression within ductular reactions in the absence of CD44 – supporting the role of a CD44-YAP axis in the control of periportal proliferation in cholestatic liver injury and protection against fibrosis development.

P1.25 ATF3 regulates liver fibrosis by promoting M1 polarization of macrophages

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Introduction Liver fibrosis is a pathophysiological proliferation of the liver connective tissue. Hepatic stellate cells (HSCs) play a central role during liver fibrogenesis and they are directly regulated by liver macrophages. Activating transcription factor 3 (ATF3) plays a key role in inflammatory reactions. However, the influence of ATF3 from bone marrow macrophages on the mechanisms of liver fibrosis has not been thoroughly investigated so far.

Methods In this study, we used bone marrow macrophage-specific ATF3 gene knockout mice to study liver fibrogenesis after intraperitoneal injection of CCl4. Firstly, we compared the degree of liver fibrosis between the cohorts. Secondly, we extracted primary mouse bone marrow macrophages and induced them to M1 and M2 phenotypes, respectively. Furthermore, we compared whether ATF3 knockdown affects the polarization of bone marrow macrophages. Finally, we co-cultured polarization-induced M1 macrophages with primary HSCs and determined the expression of α-SMA and Col1a1 in HSCs.

Results Our research shows that liver fibrosis is more severe in ATF3 knockout mice. Studies on primary mouse bone marrow-derived macrophages (BMDM) have found that knockout of ATF3 increases the expression of M1 phenotypic markers meaning that knockout of ATF3 directly targets a profibrotic phenotype. It also influences the activation of HSC while overexpression of ATF3 has the opposite effect.

Discussion There is a strong influence of ATF3 on macrophage polarization and liver fibrosis. These findings might provide some theoretical basis for new therapeutic targets during liver fibrogenesis.

Key words Liver fibrosis; macrophage; polarization; ATF3

P1.26 Characterization of a cholestasis-specific long non-coding RNAs (lncRNAs) signature in chronic liver disease

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'Cholestasis' is impaired liver bile excretion that occurs commonly in various chronic liver diseases (CLDs). Chronic cholestasis progresses to fibrosis, cirrhosis, or even fatality. Hence the early detection of cholestasis is crucial. Conventional biomarkers based on liver enzymes and serum metabolites are not sensitive enough to reliably indicate cholestasis. Long-non-coding RNAs (lncRNAs) instead have the potential in disease diagnosis especially due to their involvement in pathophysiological and physiological processes and high expression found in different cancer types. We hypothesize that lncRNAs can indicate hepatocyte changes due to cholestasis.

To elucidate this, HepG2 cells were treated with chenodeoxycholic acid (CDCA) and the Farnesoid-X receptor (FXR) agonist GW4064. Within 12 hours at subtoxic levels, cholestasis-related genes were induced, and RNA changes were analyzed via next-generation sequencing and bioinformatics statistical analysis. Four lncRNAs (HNF4-AS1, LINC02732, LINC01488, and Lnc-CCL18-68) showed significant regulation of the 14 commonly regulated lncRNAs in CDCA and GW4064 treated HepG2's. Protein coding gene pathway enrichment analysis on the REACTOME database revealed an association between lncRNAs and biological processes related to bile acid and cholesterol synthesis. Comparison with publicly available microarray dataset of cholestatic patients and other invitro models, confirmed the specificity of this signature to cholestasis.

Our study demonstrates that in vitro models can identify a unique lncRNA signature specific to liver cholestasis. Changes in lncRNA expression can be detected already under subtoxic conditions, making them potential biomarkers for early-stage CLD patients as well as other liver disease stages such as steatosis, cirrhosis to identify specific targeted therapies.

P1.27 Patient-specific hepatobiliary organoids elucidate functional alterations in intrahepatic cholestasis

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Patient-specific induced pluripotent stem cell (iPSC)-based models are a versatile resource to study pathological changes of given genetic mutations in vitro. This is of particular relevance for progressive familial intrahepatic cholestasis (PFIC), which is attributed to mutations in several genes, such as ATP8B1 (FIC1), ABCB11 (BSEP), ABCB4 (MDR3), FXR (NR1H4), MYO5B, TP2, KIF12, and where a number of variants of unknown significance (VUS) were described and where in some conditions also heterozygous variants are associated with cholestatic phenotypes.

We applied a Sendai virus based reprogramming system to generate iPSCs from patients' peripheral blood-derived cells covering compound heterozygous mutations in MYO5B, compound heterozygous mutations in ABCB11 or heterozygous mutations in ABCB11, ABCB4, and MYO5B. iPSC-derived hepatobiliary organoids from MYO5B-deficient iPSCs exhibited a reduced organoid size indicating a disturbed hepatic function and mirrored the diffuse mislocalization of the bilirubin transporter MRP2. Functional characterization of MRP2-mediated Cholyl-Lysyl-Fluorescein (CLF) and BSEP-mediated Tauro-nor-THCA-24-DBD transport demonstrated a marked reduction of transport in MYO5Bmut organoids, in comparison to unaffected control organoids. Interestingly, iPSC-based organoids derived from the patient carrying three heterozygous mutations in ABCB11, ABCB4, and MYO5B exhibited a significantly reduced BSEP-

mediated Tauro-nor-THCA-24-DBD transport, but unaltered MRP2-mediated CLF-transport.

In conclusion, iPSC-based organoid models allow functional characterization of mutations in PFIC-associated genes by assessing the hepatobiliary transport of fluorescent substrates for BSEP and MRP2, and thus, are valuable tools to functionally characterize hepatobiliary transport alterations.

P1.28 Impaired transitioning from an inactive to an active state of FXR underlies a PFIC5 phenotype

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Nuclear receptor FXR acts as a key regulator within bile homeostasis and metabolism. Within the enterohepatic cycle, reabsorbed bile acids act as agonists on FXR, which transcriptionally controls the synthesis and transport of bile acids. Binding occurs in the ligand-binding domain (LBD), favoring a conformational change to the active state in which Helix 12 interacts with the LBD to form an interaction surface for nuclear co-activators. The homozygous missense variant p.(Thr296Ile) (NM_001206979.2: c.887C>T in the FXR-encoding NR1H4 gene), identified in a PFIC5 patient (Pfister et al., 2022), is located close to this critical conformational change. The variant protein showed reduced transcriptional activity as measured via Luciferase assay on the downstream targets BSEP and SHP. Immunofluorescence staining and Western Blot assay showed normal protein localization and expression levels, indicating that the decreased transcriptional activity is indeed coupled to decreased protein activity. Using molecular dynamics simulations, we analyzed the dynamics of the conformational change from an inactive to an active state of the FXR LBD. While the wildtype protein frequently changes into the active state, this movement and the necessary perfect placement of Helix 12 was significantly impeded within the variant protein. Overall, this is the first study to sample the conformational change from an inactive to an active state within the FXR LBD and, thus, might enable critical insights into specifically targeting FXR activity. Our results, a comprehensive combination of in vitro and in silico experiments, reveal in depth the molecular mechanism of a patient-associated missense variant.

P1.29 Microbially-conjugated bile salts found in human bile activate the bile salt receptors TGR5 and FXR

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Bile salts of hepatic and microbial origin mediate inter-organ crosstalk in the gut-liver axis. Here, we assessed whether the newly discovered class of microbial bile salt conjugates (MBSCs), activate the main host bile salt receptors (TGR5 and FXR) and enter the human systemic and enterohepatic circulation. N-amidates of (chenodeoxy)cholic acid and leucine, tyrosine and phenylalanine were synthesized. Receptor activation was studied in cell-free and cell-based assays. MBSCs were quantified in mesenteric and portal blood and bile of patients undergoing pancreatic surgery. MBSCs were activating ligands of TGR5 as evidenced by recruitment of Gsα protein, activation of a cAMP-driven reporter, and diminution of LPS-induced cytokine release from macrophages. Intestine- and liver-enriched FXR isoforms were both activated by MBSCs, provided that a bile salt importer was present. Affinity of MBSCs for TGR5 and FXR was not superior to host-derived bile salt conjugates. Individual MBSCs were generally not detected (i.e. < 2.5 nmol/L) in human mesenteric or portal blood, but Leu- and Phe-variants were readily measurable in bile, where MBSCs comprised up to 213 ppm of biliary bile salts.

MBSCs activate the cell surface receptor TGR5 and the transcription factor FXR, and are substrates for intestinal (ASBT) and hepatic (NTCP) transporters. However, their entry into the human circulation is not significant. Given the low systemic levels and the excess of other equipotent bile salt species, it is unlikely that the studied MBSCs have an impact on enterohepatic TGR5/FXR signalling in humans. The origin and function of biliary MBSCs remain to be elucidated.

P1.30 A Mavs-induced type I IFN pathway contributes to non-viral liver injury upon hepatic autophagy impairment

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Hepatocellular injury drives the development of chronic inflammatory liver disease (CLD), such as alcoholic and metabolic dysfunction-associated steatohepatitis (ASH and MASH) and viral hepatitis, thereby predisposing to cirrhosis and hepatocarcinogenesis. Autophagy is an intracellular catabolic pathway that maintains cellular homeostasis serving as a prosurvival mechanism under physiological and stress conditions. Impaired autophagy has been associated with CLD pathogenesis in mouse models and patients with ASH and MASH.

We have used a genetic mouse model of liver parenchymal cell-specific autophagy impairment (ATG16L1 LPC-KO), which presents all typical phenotypic CLD characteristics, including spontaneous liver injury, hepatomegaly, hepatitis, fibrosis and eventually liver cancer. Similar to ASH and MASH patients, ATG16L1 LPC-KO mice show accumulation of cytoplasmic aggregates containing the autophagy receptor Sequestosome1/p62 in their hepatocytes. Through genetic means, we have confirmed previous studies showing that p62 partly contributes to liver injury and hepatocarcinogenesis in mice with hepatic autophagy impairment. To look for additional molecular pathways that that are relevant for hepatocellular injury in this model, we performed quantitative proteomics in livers from 8-week-old mice and our analysis revealed the upregulation of a Type I interferon (IFN) signature that was not normalized upon p62 ablation. Using genetic knockouts, we confirmed that engagement of Ifnar1 in LPCs significantly contributes to the observed liver injury, while IFN production is induced via a Mavs-dependent, but Sting-independent, pathway.

The role of these Type I IFN pathway players in liver tumor development, as well as the contribution of IFN-induced and cell death regulating protein Zbp1, are currently investigated.

P1.31 Human liver organoids as an in vitro model of fatty liver

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Introduction Liver disease research is hampered by the lack of liver models that can faithfully recapitulate complex disease phenotypes. Increasing efforts are being made to generate 3-dimensional tissue culture models, known as organoids, that adequately reflect the in vivo situation. Therefore, the aim of the present study was to generate organoids from cells isolated from resected human liver tissue (personalized model) to generate a fatty liver as an in vitro model.

Methods Human liver tissue was enzymatically dissected to obtain single cell suspensions. Liver organoids were treated with fatty acids at different time points. Cell type and activation/differentiation and fatty acid specific markers were determined by real time PCR. Finally, Paraffin blocks were prepared and organoids were further characterized by H&E and immunofluorescence staining.

Results and Conclusion Our data show that the generation of liver organoids from primary human tissues and the generation of fatty liver is possible by inducing oleic acid. However, the overall success and final differentiation status of the organoids depends on several factors, such as the enzymes used for tissue dissection, the media composition, and also the underlying disease of the patient. Nevertheless, proliferation of hepatocytes, macrophages, myofibroblasts, bile duct cells, as well as fatty acid synthase was successfully induced.

Poster Visit Session ll CLINICAL HEPATOLOGY, SURGERY, LTX 26/01/2024, 14.20pm–15.15pm

P2.01 Lower incidence of HCC and other major adverse liver outcomes in people living with HIV and chronic liver disease: a population-based cohort study

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Background People living with HIV (PLWH) show a high incidence of chronic liver disease (CLD). However, whether HIV is associated with major adverse liver outcomes (MALO) in patients with underlying CLD remains to be determined.

Methods In this population-based cohort study, data were retrieved from the Swedish National Patient Register to identify PLWH and CLD (n = 2,375) or CLD without HIV (n = 144,346) between 1997 and 2020. The cumulative incidence of MALO was calculated while accounting for competing risks (non-MALO death). Incidence rates per 1000 person-years were compared between the exposure groups (HIV vs. no HIV) with Cox regression to estimate adjusted hazard ratios (HR) and their 95 % confidence intervals (CIs).

Results The incidence rate per 1000 person-years of MALO was lower in PLWH (5.1, 95 % CI 4.2-6.1) compared to patients without HIV (13.1, 95 % CI 12.9-13.3). This translated into an adjusted HR of 0.77 (95 % CI 0.64-0.93), driven by a lower rate of hepatocellular carcinoma (adjusted HR = 0.61, 95 % CI 0.43-0.86). Consistent results were noted across a range of subgroup analyses. The 10-year cumulative incidence of MALO was lower in PLWH (5.0 %, 95 % CI 4.1-6.1) than in patients without HIV (10.9 %, 95 % CI 10.7-11.0).

Conclusion Among patients with CLD, the risk of MALO was lower in PLWH compared to those without HIV, primarily due to a lower incidence of HCC. These results suggest that HIV is not associated with a higher risk of MALO.

P2.02 Renal impairment after liver transplant is associated with indication and stabilises 1 year after transplant

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DOI 10.1055/s-0043-1777506

Background Renal impairment is frequent after liver transplant. We aimed to provide more data on the situation in Germany.

Methods We retrospectively analysed all patients who received a liver transplant at our centre between 2008 and 2019, paying attention to development of kidney function over time. Statistical standard methods were used.

Results Overall, n = 237 patients could be analysed, of whom 62 % were male. Mean age was 51.7 years at transplant. Mean estimated glomerular filtration rate (eGFR) decreased from 63 ml/min at listing to 57 ml/min at transplant; 15.6 % versus 16.3 % of patients were on dialysis at listing and transplant, respectively. While 50.6 of patient had an eGFR of at least 60 l/min at transplant, 26.6 had an eGFR between 30 to 59 ml/min and 10.1 % below 20 ml/min. At 1 year post transplant, mean eGFR had stabilised at 56.6 ml/min and the proportion of patients with eGFR below 20 ml/min or on dialysis (7.1 %) did not increase any more. While sex had no impact on kidney function after 1 year, none of 56 patients with versus 12/132 patients (9 %) without hepatocellular carcinoma were on dialysis one year after transplant (p = 0.019). Renal impairment (eGFR below 60 ml/min) was particularly frequent in alcohol-associated cirrhosis (18/28; 64 %) and rather rare in autoimmune liver disease (11/32; 34 %) and acute liver failure (2/13; 15 %).

Conclusion Impaired kidney function after liver transplant is frequent, with kidney function stabilising only 1 year after transplant. Indication for transplant has a major impact on post-transplant kidney function.

P2.03 Epimedium spp. as a trigger of acute liver failure

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DOI 10.1055/s-0043-1777507

Introduction Nowadays, people inform themselves on the internet about complementary treatments for various medical problems. Epimedium spp., ivory flowers, also known as "horny-goat weed" is heavily advertised on the internet as a remedy for erectile dysfunction, libido problems in both sexes, osteoporosis and liver disease. Potential hepatotoxicity has not been reported to date.

Case report We present a case-report of a 40-year old female patient, who presented with upper abdominal pain and jaundice (ALT 2600 U/l, AST 1863 U/l, gGT 128 U/l, total bilirubin). The initially suspected choledocholithiasis could be excluded through abdominal MRI and ERCP. In the course, the patient developed an acute liver failure with impaired hepatic synthesis and excretory function. Detailed medical history collection was unobtrusive, except for an ivory flower-based aphrodisiac. Liver biopsy revealed changes in line with a severe drug-induced liver injury with zone 3-necrosis spreading more than half of the liver parenchyma. After referral to our liver unit, the patient fulfilled the King's College criteria for non-aminoacetophen induced acute liver failure on the fifth day and was listed for liver transplantation with high urgent priority.

She was successfully transplanted after four days on the waiting list. The postoperative course remained uneventful.

Conclusion Our case report advocates for more public education about idiosyncratic hepatotoxins. The use of Epimedium spp. should be avoided due to the potential to induce severe drug-induced liver injury with fulminant acute liver failure.

P2.04 Deciphering the implications of the MAFLD and MASLD definitions in the NAFLD population: Results from a single-center biopsy study

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Background In recent years, there has been a rising trend to replace the term non-alcoholic fatty liver disease (NAFLD). Two new acronyms, metabolic (dysfunction) associated fatty liver disease (MAFLD) and metabolic dysfunction-associated steatotic liver disease (MASLD), have been proposed with unique diagnostic criteria.

Aim To investigate the applicability and agreement of the MAFLD and MASLD definitions in a population with biopsy-proven NAFLD.

Methods We conducted a retrospective analysis of prospectively collected data, focusing on patients with biopsy-proven NAFLD (n = 678; 52.7 % men) who were diagnosed and followed-up at a tertiary care center between 2009 and 2010, and from 2017 to 2023. The study patients were categorized using the diagnostic criteria for MAFLD and MASLD. To gauge the degree of agreement between the MAFLD and MASLD definitions, we used Cohen's kappa values.

Results A total of 671 (99 %) and 676 (99.7 %) patients with NAFLD met the diagnostic criteria for MAFLD and MASLD, respectively. The Cohen' kappa values for MAFLD and MASLD obtained using the NCEP-ATP III and AHA/NHLBI thresholds for waist circumference (≥ 102/88 cm and ≥ 94/80 cm, respectively) were 0.397 and 0.442, respectively.

Conclusions MASLD encompassed a slightly larger proportion of patients with biopsy-proven NAFLD compared to MAFLD. The agreement between the two definitions was found to be moderate. Additional longitudinal studies are required to shed light on the clinical implications of this terminology change.

P2.05 Exome panel diagnostics in rare childhood liver disease – good but not good enough?

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Motivation Diagnosis of rare liver diseases with genetic origin in children remains a challenging task, often due to unspecific symptoms and limited accessibility to comprehensive genetic testing. Previous work has shown efficacy of diagnosis rates for targeted gene-panel-analysis, using preselected patient cohorts based on phenotypes, mainly in cholestatic diseases. The aim of this work was to evaluate this efficacy for a heterogenous patient group with different phenotypes throughout all age subgroups.

Methods 56 patients (24 female, mean age: 47,5 ± 69,3 months) were evaluated for unexplained liver disease in 11 pediatric hepatology centers in Germany. All underwent high-throughput-sequencing (targeted exome-panel-analysis performed at CeGaT Tuebingen between 2014-2020) with clinical interpretation of up to 128 genes. We subdivided this cohort according to the degree of phenotypic characterization before sequencing: I: phenotype associated with specific suspected clinical diagnosis; II: phenotype likely associated with specific group of diseases (e.g., PFIC); III: no association.

Results The diagnostic yield was 53,6 %. Upon identification of a specific genotype in this group, a therapeutic concept could be established. The most common diagnosis was PFIC (n = 10). The diagnostic yields in subgroups were I: 64 %; II: 42 %; III: 57 %.

Conclusion The diagnostic yield was comparable to previous studies even though a heterogenous group without preselection was evaluated. We show that exome-panel-analysis can be effective even for patients in which phenotyping does not allow association to a specific suspected disease pre-genotyping. In the future (trio-)whole-genome-sequencing and/or transcriptome-sequencing may lead to further improve the diagnostic yield and potentially identify new disease-causing genes.

P2.06 The Freiburg Index of Post-TIPS Survival (FIPS) identifies patients with further decompensation after transjugular intrahepatic portosystemic shunt implantation: a multicenter observational study

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Introduction The Freiburg Index of Post-TIPS Survival (FIPS) defines a high-risk group of patients with significantly impaired survival following TIPS implantation. As further decompensation is significantly associated with reduced survival, we hypothesized that further decompensation may be an important hallmark in FIPS high-risk patients after TIPS.

Methods 1514 cirrhosis patients allocated to TIPS implantation for treatment of refractory ascites or secondary prophylaxis of variceal bleeding from eight centers were retrospectively included. Primary outcome was further decompensation within 90 days after TIPS. Secondary outcomes were the development of acute-on-chronic liver failure (ACLF) within 90 days and one-year transplant-free survival.

Results The cumulative incidence of further decompensation, with death and liver transplantation as competing risks, was significantly higher in FIPS high-risk patients compared to low-risk patients (0.56 vs. 0.36). Moreover, the cumulative incidence of ACLF within 90 days after TIPS was markedly increased in high-risk patients (0.37 vs. 0.11). Uni- and multivariable competing risk-regression analyses confirmed that FIPS high-risk classification was an independent predictor of further decompensation (SHR 1.768, 95 % CI 1.454 – 2.149, p < 0.001) and ACLF after TIPS (SHR 1.943, 95 % CI 1.276 – 2.958, p = 0.002). Further decompensation and the development of ACLF after TIPS were associated with significantly reduced transplant-free survival.

Conclusions The present study reveals that the FIPS predicts further decompensation after TIPS implantation, which might explain impaired survival in FIPS high-risk patients. Therefore, tailored clinical management strategies including early evaluation for liver transplantation for FIPS high-risk patients should be considered.

P2.07 Soluble urokinase plasminogen activator receptor (suPAR) levels predict survival in patients with portal hypertension undergoing TIPS

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Background Transjugular intrahepatic portosystemic shunt (TIPS) is an effective therapy for complications of portal hypertension. Nonetheless, the clinical courses following TIPS insertion decisively differ between patients and the identification of the ideal candidates remains challenging. We evaluated the potential clinical relevance of circulating suPAR, a marker of immune activation that has previously been associated with liver inflammation, in patients receiving TIPS insertion.

Methods suPAR concentrations were measured by ELISA in hepatic vein (HV) and portal vein (PV) blood from 99 patients as well as in peripheral venous samples from 150 additional patients undergoing TIPS insertion. The association of suPAR concentrations with outcome was assessed by Kaplan-Meier methods and Cox regression analysis.

Results suPAR concentrations were significantly higher in HV samples compared to PV samples and correlated with the presence of ascites, kidney injury, and consequently with the Child-Pugh and MELD score. Patients with lower suPAR levels had a significantly better short- and long-term survival following TIPS insertion, which remained robust after adjustment for confounding factors in multivariate Cox-regression analysis. Sensitivity analysis demonstrated an improvement of risk prediction in patients stratified for the Child-Pugh or MELD score. In an independent validation cohort, higher suPAR concentrations in peripheral vein blood indicated poor transplant-free survival after TIPS, particularly in patients with Child-Pugh A/B cirrhosis.

Conclusion suPAR derives in large parts from the injured liver and its concentrations predict outcome in patients undergoing TIPS. suPAR as a surrogate of hepatic inflammation may be used to stratify care in patients following TIPS insertion.

P2.08 Oxaliplatin-induced spleen hypertrophy as a predictor of liver-specific complications following curative resection of colorectal liver metastases.

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Introduction In patients with colorectal liver metastases (CRLM), preoperative chemotherapy may cause sinusoidal obstruction syndrome, portal hypertension, and splenomegaly. This study evaluates the association between splenic hypertrophy and postoperative complications, after major resection for CRLM.

Method This retrospective study included patients with CRLM, who underwent major curative liver resection following chemotherapy between 2010-2021. Volumetric measurements of the spleen were performed on preoperative, pre- and post-chemotherapy CT and MRT images, using segmentation software (3D-Slicer). Receiver-operating characteristic analysis was performed to determine the optimal spleen hypertrophy cut-off for predicting postoperative liver-specific complications. These included biliary complications, haemobilia, post-hepatectomy liver failure, hepatorenal syndrome, postoperative hemorrhage due to inadequate coagulation factor synthesis, and portal-hypertension-related complications, such as ascites. Risk factors regarding postoperative liver specific complications were examined using logistic regression.

Results Of 115 patients included in the study, 78 (68 %) received oxaliplatin. A threshold of 8.6 % splenic hypertrophy (Youden Index = 0.25) was identified as a predictor of liver-specific complications (AUC 0.623; p = 0.022). Patients above the cut-off (n = 62) were administered oxaliplatin significantly more often (84 % vs. 49 %; p < 0.001). Rates of liver fibrosis (58 % vs. 42 %, p = 0.029) and liver-specific complications (63 % vs. 38 %, p = 0.007) were also higher in that group. Multivariable logistic regression analysis for liver-specific complications, showed an odds ratio of 2.86 (95 %CI 1.104-7.402, p = 0.03) for splenic hypertrophy above 8.6 %.

Conclusion Preoperative splenic volumetry may be a valuable predictor of postoperative liver-specific complications in patients undergoing CRLM resection after chemotherapy. Further studies are necessary to investigate the impact on a larger cohort.

P2.09 Body composition and bone mineral density in patients with advanced liver cirrhosis

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Malnutrition is common in patients with advanced stages of liver cirrhosis, eventually leading to alterations of body composition (BC) and further complications. In this study, we analyzed prevalence and prognostic impact of impaired bone mineral density (BMD) and loss of muscle mass in patients with liver cirrhosis and portal hypertension.

BMD of 116 patients with liver cirrhosis allocated to transjugular intrahepatic portosystemic shunt (TIPS-) implantation was analyzed by dual-energy X-ray absorptiometry (DXA). Additionally, BC was assessed by DXA in 107 patients. Impaired BMD was defined by a T-score ≤ -1, osteoporosis by a T-score ≤ -2.5. An appendicular skeletal muscle index < 7.26 (men) and < 5.45 (women) defined sarcopenia.

Most patients had advanced liver cirrhosis with a Child-Pugh-Stadium B (63.9 %) or C (19.7 %). 48.6 % were sarcopenic with a predominance of male patients (58.7 % vs. 25.0 %, p = 0.001). Moreover, 69.8 % had impaired BMD and 34.5 % osteoporosis. Alcohol consumption and presence of ascites were not associated with sarcopenia or impaired BMD. Lower body mass index (BMI) was an independent risk factor for both (sarcopenia: OR 0.751, p < 0.001; impaired BMD: OR 0.849, p < 0.001). Additionally, male sex was an independent risk factors for sarcopenia (OR 11.484, p < 0.001). Impaired BMD, but not sarcopenia, negatively influenced 12-month survival after TIPS-implantation (HR 2.573, p = 0.033).

Sarcopenia and impaired BMD are very common in patients with advanced liver cirrhosis and represent risk factors for complications and death. Therefore, assessment of nutritional status and specific treatment should be included in clinical practice.

P2.10 Preoperative C-reactive-protein-to-albumin ratio as a predictor of patient- and graft-survival after deceased-donor liver transplantation

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Background Advances in immunosuppression have improved outcomes after deceased-donor liver transplantation (DDLT). However, patient factors still affect long-term survival and graft loss. This study examines the predictive value of preoperative C-reactive-protein-to-albumin ratio (CAR) regarding recipient survival (RS) and graft survival (GS) after DDLT.

Materials and methods This study included DDLT recipients between 2010-2023. Re-transplantations, split-liver transplantations and patients dying within 90 days were excluded. Receiver operating characteristic curve (ROC), area under the curve (AUC) and Youden Index (YI) analyses were used to define a CAR cut-off for the prediction of RS and GS. Survival analyses were carried out using Kaplan-Meier, log rank test, and Cox regression.

Results The 444 included patients had a mean RS of 110 months (95 %CI 105-116 months) and GS of 106 months (95 %CI 100-112 months). The best predictive ability of CAR was shown for 3-year RS (3YRS, AUC = 0.67, p < 0.001) and 3-year GS (3YGS, AUC = 0.65, p < 0.001), with CAR = 41 % being the best cut-off for both outcomes (YI 0.35 and 0.32, respectively). Patients with CAR ≥ 41 % had significantly lower mean 3YRS (30 vs. 34 months, p < 0.001) and 3YGS (29 vs. 33 months, p < 0.001). Cox regression analysis for CAR ≥ 41 % showed a hazard ratio of 3.78 (95 % CI 2.19-6.53, p < 0.001) for 3YRS and 3.11 (95 % CI 1.93-5.01, p < 0.001) for 3YGS.

Conclusion Higher preoperative CAR is associated with inferior patient and graft survival, and may serve as an additional tool for identifying patients at risk after DDLT.

P2.11 A comparison of clinical risk scores for survival prediction after curative-intent resection of colorectal liver metastases.

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Introduction Clinical risk scores (CRS) have been developed to predict overall survival (OS), after liver resection for colorectal liver metastases (CRLM). In this study, we compare 11 previously described CRS on a monocentric cohort.

Method This retrospective study included patients, who underwent liver resection for CRLM between 2010-2021. Recurrent metastases, explorative laparotomies and patients dying within 90 days of surgery were excluded. The following 11 CRS were assessed: Fong, Nordlinger, Nagashima, Konopke, Basingstoke Predictive Index (BPI), tumour burden score (TBS), resection severity index (RSI), Kulik, RAS-mutation-CRS, modified TBS (mTBS), genetic and morpholigal evaluation (GAME) score. Survival was analysed using Kaplan-Meier and the log-rank test. Predictive abilities were evaluated using the Akaike-Information-Criterion (AIC), Harrell's-C-Index, and AUC-analyses.

Results Median OS for 528 included patients was 26 months (95 % CI 23-28 months). Apart from RSI (p = 0.570), all other CRS could stratify patients according to predicted OS: Fong, p = 0.007; Nordlinger, p < 0.001; GAME-score, p = 0.006; Nagashima, p < 0.001; Konopke, p < 0.001; BPI, p < 0.001; TBS, p < 0.001; Kulik, p < 0.001; RAS-mutation-CRS, p < 0.001; mTBS, p < 0.001. mTBS consistently performed within the top 3 scores for OS (AIC 1725, C-Index 0.61), while TBS (AUC = 0.654, p = 0.001) and mTBS (AUC = 0.62, p < 0.001) were the best for 1-year- and 5-year-survival, respectively.

Conclusion Of 11 externally developed and validated CRS, the mTBS was consistently among the best, in terms of OS prediction. Further studies are neccesary to elucidate the effect of factors such as tumor biology.

P2.12 Circulating thrombospondin-2 as a novel, highly predictive serum marker of liver fibrosis and outcome in patients with primary sclerosing cholangitis

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Thrombospondin-2 (TSP2) is a matricellular protein in organ fibrosis. TSP2 expression is highly upregulated in fibrogenesis, as shown in patients with fatty liver disease, lung fibrosis and in a pilot study of primary sclerosing cholangitis (PSC), where a wheat free diet reduced elevated TSP2 levels. We therefore hypothesised that TSP2 may serve as a novel marker of fibrosis in PSC.

115 PSC patients were selected from the biobank of our tertiary care centre and included in the study. All patients had their serum TSP2 levels determined by our validated inhouse sandwich ELISA. Liver stiffness was measured by transient elastography (TE). In addition, parameters of disease activity and patient outcome were assessed.

Baseline serum TSP2 level showed a strong positive correlation with liver fibrosis, as determined by TE (r = 0.631, p < 0.001). The AUROC values for TSP2 in predicting advanced liver stiffness (≥ F3) was 0.83. This value was better than those of FIB-4 index (0.76) and APRI (0.77). Over a median follow-up of 70 months, 12.2 % of patients underwent liver transplantation. Patients with baseline serum TSP2 levels indicating advanced fibrosis (≥ 73.1ng/ml) had significantly reduced transplant-free survival (Log Rank p < 0.001).

Circulating TSP2 levels were highly associated with the presence of advanced fibrosis and the transplant-free survival, suggesting its potential as a prognostic biomarker for predicting the efficacy of specific therapies in patients with PSC. Further studies to validate the power of serum TSP2 to predict the outcome and the effect of therapeutic interventions in

P2.13 Analytical methods used in creatinine measurement significantly impact Model for End Stage Liver Disease score: Is there a need for modification?

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Background The Model for End Stage Liver Disease (MELD) score is utilized as a prognostic tool for end-stage liver disease. The variations in laboratory tests have a substantial impact on MELD. For creatinine as one parameter in the MELD score, measurements are mostly performed by either Jaffé kinetic or enzymatic assays. In both assays, bilirubin is recognized to significantly interfere with the creatinine detection.

Aim Our objective was to characterize the bias of bilirubin interference on creatinine for both methods employing Roche cobas6000 clinical chemistry analyzers. Secondarily, we aimed to investigate the impact of this interference on MELD.

Methods We set up two dilution matrices using water and PBS as solvents containing creatinine ranging from 0 to 5 mg/dL and bilirubin ranging from 0 to 35 mg/dL. The effect of bilirubin on creatinine levels was modeled by three-dimensional regression analysis for both methods separately. Subsequently, the models were used to recalculate MELD scores from retrospective laboratory results (N = 13,186) from patients with liver cirrhosis.

Results With increasing bilirubin, creatinine by Jaffé was increased, while creatinine by enzymatic method was decreased. Recalculation of MELD scores using corrected creatinine values yielded a significant number of MELD scores which were up to two points lower for Jaffé (N = 564, 3.5 %) and up to three points higher for enzymatic assay (N = 1,016, 7.7 %).

Conclusion The method-dependent interference of bilirubin on creatinine significantly impacts MELD. For the calculation of standardised MELD scores, correction of creatinine values accounting for both methods and bilirubin effect is required.

P2.14 Insufficient control of cholestatic pruritus in primary biliary cholangitis (PBC) with current therapies: preliminary baseline data from the ongoing Phase 3 GLISTEN study

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Background The Phase 2b GLIMMER study that assessed linerixibat, an ileal bile acid transporter inhibitor, for treatment of cholestatic pruritus in 147 patients with PBC, demonstrated that 62 % of patients experienced previous treatment failures, and 38 % still experienced pruritus despite concomitant anti-pruritus therapy at baseline. This blinded preliminary analysis of baseline data from GLISTEN further explores the utilization of available pruritus therapies in a controlled PBC population.

Method GLISTEN (NCT04950127) is an ongoing Phase 3 study investigating the efficacy and safety of linerixibat in patients with PBC and moderate-severe pruritus. Pruritus severity is measured using a 0–10 numerical rating scale; stable concomitant pruritus therapy is allowed.

Results Data from 154 patients were included in this preliminary analysis. At baseline, 94 % of patients were female, mean (standard deviation, SD) age was 56.0 (11.6) years, 11 % had compensated cirrhosis. Mean (SD) alkaline phosphatase was 225.6 (164.4) IU/L and mean (SD) total bilirubin was 11.64 (8.0) µmol/L. 56 % of patients had severe pruritus, 48 % were receiving concomitant pruritus therapy: only 6 % were receiving concomitant bile acid-binding resins despite being recommended as first-line therapy, while 22 % were receiving fibrates, 8 % selective serotonin reuptake inhibitors, 5 % gabapentin, 5 % antihistamines, 3 % nalfurafine, 3 % naltrexone, 3 % rifampicin, and 1 % pregabalin.

Conclusion Despite receiving licensed and/or off-label therapies, including fibrates, patients with PBC still experience moderate-severe pruritus. These data support previous findings that treating pruritus remains a high unmet need and more effective therapies are required.

Funding GSK (212620).

P2.15 Lactate metabolism in decompensated liver cirrhosis

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Lactate is a metabolic product of anaerobic glycolysis that is cleared by the liver and is therefore an important diagnostic parameter in patient management. Patients with liver cirrhosis are characterised by elevated lactate levels, suggesting impaired hepatic lactate clearance.

To further analyse the hepatic lactate clearance in cirrhosis, lactate levels as well as glucose, oxygen content and pH were analysed in 25 patients in peripheral and central venous blood as well as blood collected from the hepatic, portal, mesenteric and splenic veins during transjugular intrahepatic portosystemic shunt (TIPS) procedures.

Lactate concentration in central venous blood was significantly increased in patients with Child-Pugh stage C liver cirrhosis compared with stages A and B cirrhosis. In this respect, the data indicate that the most important net lactate producers are the muscles and the gastrointestinal tract, as shown by the lactate concentrations in the peripheral venous blood and the mesenteric vein, respectively, whereas the cirrhotic liver is not a net lactate producer.

Interestingly, however, analysis of portal and hepatic vein lactate concentrations suggested that the metabolic capacity and consequent lactate clearance was not affected by the stage of liver cirrhosis. Instead, the transient increase in lactate concentration after initiation of TIPS could be explained, at least in part, by transiently impaired clearance by direct shunting of blood from the mesenteric vein through the intrahepatic stent.

In conclusion, elevated lactate levels in patients with cirrhosis are more likely to be due to microcirculatory disturbances and consequent increased anaerobic glycolysis than to decreased clearance.

P2.16 Role of hypersplenism and transjugular intrahepatic portosystemic shunt implantation on cytopenia in decompensated liver cirrhosis

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Cytopenia in patients with cirrhosis is a common but incompletely understood complication. It has been discussed that portal hypertensive hypersplenism is causative, while the results of studies investigating the effect of reducing hypersplenism have been conflicting. However, the fact that substitution of thrombopoietin analogues improved thrombocytopenia suggests that the observed reduction of thrombopoietin in cirrhotic patients plays a crucial role. The aim of the present study was to investigate the effects of reducing portal hypertension by implanting a transjugular intrahepatic portosystemic shunt (TIPS) on haematopoiesis and to further clarify the role of hypersplenism in cytopenia.

In 70 patients, haematological parameters were analysed before and after the TIPS procedure and correlated with liver synthesis parameters and functional parameters like spleen size and splenic elastography. Moreover, the number of blood cells in blood drawn from portal vein, splenic vein and inferior vena cava during the procedure was analysed.

The analysis of cell count in portal and splenic vein suggest that hypersplenism does not appear to play a major role in thrombocytopenia, but does play a role in anaemia. The latter improves as does thrombocytopenia after TIPS implantation, although stent-related haemolysis is already observed during the procedure and can also be assessed in the longer term. Whether TIPS implantation and recompensation also affect haematopoiesis, as measured by thrombopoietin and erythropoietin, is currently being investigated.

In conclusion, these data show that TIPS has a beneficial effect on cytopenia as a side effect and that hypersplenism is only potentially relevant in the context of anaemia.

P2.17 Impact of thiamine (vitamin B1) supplementation on hepatic encephalopathy and mortality in patients with decompensated alcoholic liver cirrhosis

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DOI 10.1055/s-0043-1777521

Background Malnutrition and cognitive impairment are common complications in patients with decompensated alcoholic liver cirrhosis (LC). Therefore, some guidelines recommend thiamine substitution in these patients. However, little is known about its effect on hepatic encephalopathy (HE) or other liver-related complications. We evaluated the frequency of thiamine supplementation in patients with decompensated alcoholic LC and its impact on HE development and transplant-free survival.

Methods We retrospectively investigated 289 patients with decompensated alcoholic LC who were admitted to our hospital between 2011 and 2023 with at least one follow up in our clinic. Medication at discharge was screened for thiamine-containing supplements. Time to first HE and transplant-free survival was examined during 90 days after discharge.

Results A number of 139 (48.1 %) patients received daily thiamine-containing supplements, most frequent was intake of vitamin-B-complex (52.5 %) or single vitamin-B1 (47.5 %). Less patients (8.6 %) took multivitamin-supplementation. No significant differences regarding sex, MELD, HE at baseline and intake of HE-prophylaxis between patients with and without thiamine-supplementation were observed.

Overall, 69 patients developed HE, 24 died and six received LTx. Multivariable Cox regression analysis adjusted for MELD, age and sex indicates no difference regarding transplant-free survival between groups (HR = 0.91; p = 0.80). Furthermore, Competing Risk analysis, additionally adjusted for HE at baseline and intake of HE-prophylaxis, shows no significant difference between groups concerning risk of HE development (HR = 0.87; p = 0.56).

Conclusion As thiamine does not ameliorate risk of HE development or death in decompensated cirrhosis, further studies are needed to reflect benefits and possible risks, e.g. polypharmacy, of broad vitamin-B application.

Poster Visit Session lll METABOLISM (INCL. MASLD) 26/01/2024, 16.25pm–17.00pm

P2.18 Methylprednisolone-induced liver injury – an emerging autoimmune-mediated disease

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Introduction Methylprednisolone-induced liver injury affects patients with high-dose pulse treatments with methylprednisolone. Patients suffering from autoimmune diseases, in particular multiple sclerosis, are a risk group. Here we present a case series of 8 liver biopsies of patients with methylprednisolone-induced liver injury that were diagnosed at our department.

Material and methods We included 8 core needle liver biopsy samples that were examined between 2018 and 2023. 7 patients had a past medical history of either multiple sclerosis or acute disseminated encephalomyelitis, having received high dose pulse treatment with methylprednisolone within the last few weeks. All patients were biopsied following abnormal liver function tests without any apparent clinical cause. Routine liver biopsy stainings were performed after formalin fixation and paraffin embedding. Ancilliary testing with immunohistochemistry and molecular pathology was done in select cases.

Results The severity of histopathologic liver injury in our cohort ranges from slight to moderate. Biopsies from all patients showed a hepatitic injury pattern with single hepatocyte necrosis and mild to moderate inflammation of the acinia and portal tracts with a predominance of lymphocytes. Viral infection was ruled out in all patients. A diagnosis of drug-induced liver injury was made in all cases.

Conclusions Methylprednisolone-induced liver injury represents an emerging disease. The histopathologic liver injury pattern is consistent with drug-induced liver injury. The mechanism is still unclear. Previous studies suggest an autoimmune-mediated liver injury caused by methylprednisolone. It seems likely that the association of liver injury with a strong immunosuppressant as causative drug might lead to misdiagnosis.

P2.19 Ultraschall-definierte Sarkopenie ist ein unabhängiger Prädiktor für akute Dekompensation und ACLF bei chronischer Lebererkrankung

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Einleitung Sarkopenie ist eine häufige und unterschätzte Komplikation bei Patienten mit chronischer Lebererkrankung. Die frühzeitige Diagnose der Sarkopenie ist bei diesen Patienten entscheidend, da sie ein erhebliches Risiko für die Gesamtmortalität darstellt.

Die von der EASL empfohlene Standardmethode ist die CT-graphische Bestimmung des Skelettmuskelindex (SMI) bei LWK3.

Ziele In dieser Studie soll der Zusammenhang zwischen US-definierter Sarkopenie und akuter Dekompensation (AD) und Mortalität bei Patienten mit chronischen Lebererkrankungen untersucht werden.

Methodik 63 Patienten mit chronischer Lebererkrankung und klinisch signifikanter portaler Hypertension wurden eingeschlossen. Primärer Endpunkt waren die AD und das Akut-auf-chronische Leberversagen innerhalb eines Jahres. Die ventrale Muskeldicke der Oberschenkelmuskulatur wurde mittels Linearschallkopf gemessen und ein Cut-off-Wert für die Muskeldicke zur Definition von Sarkopenie bestimmt.

Ergebnisse Insgesamt 44 % der 63 Patienten hatten eine äthyltoxische Zirrhose. Eine AD wurde bei 15 (24 %) der Patienten beobachtet.

Eine US-definierte Sarkopenie konnte bei 36 (57 %) der Patienten nachgewiesen werden. Sarkopene Patienten hatten einen signifikant höheren MELD Score (15 vs 11, p = 0.005) sowie ein höheres Alter (63 vs 52 Jahre, p = 0.001). Außerdem wiesen sie eine fast 10-fach höhere Inzidenz von AD (39 % vs. 3,7 %, p = 0,001) sowie eine höhere Gesamtmortalität innerhalb eines Jahres (28 % vs 3,7 %, p = 0,013) auf. Das Vorhandensein einer Sarkopenie war neben MELD und Child Pugh ein unabhängiger Prädiktor für das Auftreten einer AD (aHR 16,55 [2.01-136.02]; p = 0.009).

Schlussfolgerung In dieser Studie konnte US-definierte Sarkopenie als unabhängiger Prädiktor für das Auftreten einer AD sowie des Versterbens innerhalb eines Jahres definiert werden. Die Ergebnisse konnten Ultraschall als sensitive Methode zur Diagnostik der Sarkopenie herausstellen.

P2.20 Automated assessment of three-dimensional TIPS geometry predicts shunt dysfunction in decompensated liver cirrhosis

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Background and aims In selected patients, complications of portal hypertension like refractory ascites or variceal bleeding can be treated with a transjugular intrahepatic portosystemic shunt. However, a notable problem in this group of patients is TIPS dysfunction. This study investigated the prognostic value of three-dimensional (3D) TIPS geometry for the development of TIPS dysfunction.

Methods One hundred and eight patients (n = 108) undergoing TIPS procedure between 2014 and 2019 were analyzed in this monocentric retrospective study. CT reconstructions were used to assess 3D TIPS geometry. Parameters of 3D TIPS geometry were calculated using a semi-automated algorithm. Additionally, 2D angiograms were reviewed to determine 2D geometric characteristics of the TIPS. Primary outcome was development of TIPS dysfunction defined by need for TIPS revision.

Results A total of 32 patients developed TIPS dysfunction and were compared to the dysfunction-free 76 patients. In terms of 3D TIPS geometry parameters, the position of the cranial stent end in the hepatic vein (p = < 0.001, HR 1.069, 95 % CI 1.039-1.101) and the stent curvature (p = 0.001, HR 1.023, 95 % CI 1.009-1.037) were significantly associated with the development of TIPS dysfunction in a multivariate Cox regression analysis. Of note, none of the 2D TIPS geometry parameters were significantly associated with TIPS dysfunction.

Conclusion This monocentric study demonstrates the prognostic value of 3D TIPS geometry. A more pronounced stent curvature and a longer distance of the cranial stent end in the hepatic vein to the Vena cava inferior were independent predictors of TIPS dysfunction.

P2.21 Auswirkungen des Alkoholkonsums auf den Leberphänotyp von Personen mit Alpha1-Antitrypsin-Mangel

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Einleitung/Ziele Da Alpha1-Antitrypsin-Mangel (AATM) das Risiko einer Lebererkrankung erhöht, haben wir untersucht, inwiefern Alkoholkonsum den Leberphänotyp bei Erwachsenen mit der heterozygoten/homozygoten Pi*Z-Variante (Pi*MZ/Pi*ZZ-Genotyp) in der United Kingdom Biobank (UKB) und dem europäischen Alpha1-Leberkonsortium modifiziert.

Methodik Von 17 145 Pi*MZ- und 141 Pi*ZZ-Personen sowie 425 002 Nicht-Trägern (Pi*MM) aus der UKB wurden anamnestische Angaben des Alkoholkonsums ausgewertet. 561 Pi*ZZ-Probanden aus dem europäischen Alpha1-Leberkonsortium wurden untersucht und einer Messung von Carbohydrate Deficient Transferrin (CDT) unterzogen. Ergebnis: Ein schädlicher Konsum (Frauen ≥ 40 g/d, Männer ≥ 60 g/d) wurde selten angegeben (~1 %). Über 80 % der Personen gaben an, keinen/wenig Alkohol zu konsumieren. Bei UKB Pi*MM/MZ-Probanden führte ein mäßiger Verzehr (Frauen 12–39 g/Tag, Männer 24–59 g/Tag) zu einem < 30 %igen Anstieg in den erhöhten Transaminasen. Der Effekt auf die abnormalen GGT-Werte war ausgeprägter (Pi*MM: 15,0 % vs. 23,0 %; Pi*MZ: 15,7 % vs. 22,5 %), aber in beiden Gruppen vergleichbar. Bei beiden Genotypen führte schädlicher Alkoholkonsum zu einem mindestens zweifachen Anstieg des Anteils an Probanden mit erhöhten Transaminasen, GGT-Spiegeln sowie erhöhter AST/Thrombozyten-Ratio (APRI). Moderater Alkoholkonsum hatte in beiden Pi*ZZ-Kohorten keine offensichtliche Auswirkung auf die Transaminasen, GGT-Werte waren zahlenmäßig häufiger erhöht. Die Pi*ZZ-Probanden aus der europäischen Kohorte wiesen mit moderatem Konsum tendenziell höhere CAP-Werte als Steatose-Marker, aber keine Unterschiede in der Lebersteifigkeit auf. 14 % der Probanden hatten erhöhte CDT-Serumspiegel (≥ 1,7 %), welche in der univariablen Analyse höhere GGT-Spiegel (72,5 vs. 60,0 % ULN, p = .011) sowie höhere APRI-Scores (0,36 vs. 0,30, p = .006) aufwiesen.

Schlussfolgerung Die Genotypen Pi*MZ/Pi*ZZ scheinen die hepatische Toxizität von moderatem Alkoholkonsum nicht merklich zu verschärfen.

P2.22 Turmeric-induced acute liver failure – A case report of an idiosyncratic drug-induced liver injury (DILI)

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DOI 10.1055/s-0043-1777526

Background and Aims The increasing use of herbal products and dietary supplements, such as turmeric, has raised concerns about potential hepatotoxicity. Turmeric recognized for its antioxidant properties and frequently used for its anti-inflammatory effects, has been linked to rare cases of acute liver failure. We present a case of a 43-year-old female patient exhibiting symptoms including jaundice, discoloured stool, brown urine, pruritus, fatigue, and abdominal pain.

Method We conducted continuous laboratory assessments of transaminases, cholestasis parameters, and coagulation status. Additionally, abdominal ultrasound examination and Doppler ultrasonography were performed. We ruled out other potential causes of acute liver failure through anamnestic, laboratory, and microbiological investigations.

Results We assessed ALT, ALP, and bilirubin levels upon admission, revealing a mixed pattern of elevated liver enzymes consistent with drug-induced liver injury (DILI) (AST 3166 U/l, ALT 3152 U/l, GGT 398 U/l, AP 396 U/l, bilirubin 6.3 mg/dl). The patient had no history of alcohol consumption, drug use, trauma, family liver disease, or regular medication. The patient reported using turmeric pills. Liver perfusion ultrasound showed no abnormalities, and laboratory and microbiological analyses ruled out autoimmune, infectious, metabolic, genetic and cholestatic causes of acute liver failure. Thus, the diagnosis of drug-induced liver failure was made, and prednisolone therapy was initiated, leading to rapid clinical improvement and complete recovery.

Conclusion The widespread use of dietary supplements, such as turmeric, necessitates evaluation for potential links to severe hepatic damage. We highlight the diverse liver injuries associated with herbal remedies, impacting different hepatic and biliary cells.

P2.23 Cholestatic Hepatitis as a Rare Manifestation of Infectious Mononucleosis

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DOI 10.1055/s-0043-1777527

Background Infectious mononucleosis, caused by Epstein-Barr virus (EBV) infection, typically presents with fever, lymphadenopathy, and tonsillitis. Worldwide, EBV seroprevalence in adults exceeds 90 %. Primary infections in childhood are typically asymptomatic, while adult primary infections result in classic infectious mononucleosis symptoms in 30-60 % of cases. Involvement

of other organ systems, including the liver, is less common but possible. Most patients exhibit slight increases in transaminases, and jaundice occurs in only 5 % of cases.

Case report In December 2022, a 22-year-old nurse presented with profound jaundice and itching, following an EBV infection diagnosed in October. Despite only mildly elevated transaminases, and increased AP but normal GGT, the patient developed hyperbilirubinemia up to 23.7 mg/dL without other liver function impairments. Laboratory workup was negative for viral hepatitis A-E and autoimmune liver markers (AIH, PBC, PSC). MRCP showed no signs of bile duct pathology. Liver histology initially suggested a possible autoimmune hepatitis (AIH) episode, leading to the initiation of corticosteroid therapy after ruling out active EBV. However, the patient showed no improvement, prompting discontinuation of corticosteroid treatment.

Upon second evaluation of the liver biopsy, EBV hepatitis was confirmed instead of AIH. Additionally, benign recurrent intrahepatic cholestasis (BRIC) and progressive familial intrahepatic cholestasis (PFIC) were ruled out in immunohistochemistry and genetic testing. Within 3 months hyperbilirubinemia resolved spontaneously.

Conclusion This case emphasizes the complexity of distinguishing the atypical presentation of EBV infection from rare conditions and the importance of comprehensive evaluation in cases of cholestatic hepatitis.

P2.24 An innovative interprofessional approach to therapeutic drug monitoring in liver failure improves guideline adherence and quality of care

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DOI 10.1055/s-0043-1777528

Background & Aims Acute-on-chronic liver failure (ACLF) patients urgently need effective treatments for primary triggers like bacterial infections. Meropenem is essential for severe infections, and its dosage is optimally controlled using therapeutic drug monitoring (TDM). We investigated the outcomes of TDM for meropenem in ACLF patients.

Methods In a medical ICU practicing an interprofessional culture, the TDM outcomes for meropenem were evaluated in 25 ACLF patients. An interprofessional team of physicians, pharmacists, and nurses implemented the approach. TDM was done weekly using high-performance liquid chromatography. Meropenem serum levels and team recommendations were the main outcomes, with a seasonally adjusted period as control.

Results Initial TDM in the 25 patients showed a mean meropenem serum concentration of 20.9 ± 9.6 mg/l, with 84.0 % exceeding the target range. All dosing recommendations by the interprofessional team were implemented, leading to a 10.0 % decrease in meropenem application compared to the control.

Conclusion The interprofessional TDM approach significantly optimized meropenem dosing in ACLF patients, ensuring patient safety and reducing meropenem use. Such an approach is crucial for effective carbapenem application in ICU settings.

P2.25 Therapeutic plasma exchange as a bridge to recovery for acute liver failure due to Amanita phalloides poisoning

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DOI 10.1055/s-0043-1777529

The ubiquitous availability of digital media does not only have advantages. Inexperienced mushroom pickers increasingly frequently use APPs for mushroom identification and consume forest mushrooms with false security. Currently, for example, life-threatening Amanita phalloides poisoning is becoming more frequent.

A 65-year-old patient presented with a latency period of approximately 40 hours after consumption of the mushrooms. Nausea and vomiting, as well as loss of strength and cramps in arms and legs, had already been present for about 24 hours. Laboratory tests revealed coagulation failure and markedly elevated transaminases (INR 2.1, GOT 2590 U/l, GPT 3119 U/l). The Meld Score was 33. Only when asked explicitly the patient reported eating wild mushrooms he had collected. The vomit and mushroom debris were recovered and immediately examined by a mushroom expert. Immediately after admission to our intensive care unit, liver protective therapy with Silibinin and N-Acetylcysteine was initiated.

Analysis by a fungal expert revealed evidence of Amanita phalloides. At an INR of 3.3 with massive diffuse skin hemorrhages and rising transaminases (GOT 4825U/l, GPT 4104U/l), repeated therapeutic plasma exchange (exchange of 1.3 times the plasma volume) was performed to control the complications of liver failure. In the course, transaminases and coagulation parameters stabilized. The patient is now in excellent general health.

Our patient case shows that early diagnosis, as well as therapy, are crucial to reducing the lethality of tuberous leaf fungus poisoning. As a bridging therapy for the stabilization and regeneration of liver function, therapeutic plasma exchange is an excellent option.

P2.26 First description of a genetic variant in Semaphorin 7A in a case of intrahepatic cholestasis of pregnancy

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Hereditary intrahepatic cholestasis encompasses a spectrum of clinical phenotypes including intrahepatic cholestasis of pregnancy (ICP), low phospholipid-associated cholelithiasis (LPAC) or progressive familial intrahepatic cholestasis (PFIC). In the late 1990s, genetic variants in ATP8B1, ABCB11, and ABCB4 were described to be associated with hereditary cholestasis. Next generation sequencing techniques led to the identification of further cholestasis-related genes.

After recurrent miscarriages, a woman presented in her third pregnancy at week 30 suffering from severe pruritus despite normal serum bile acids (7.8µmol/l, [< 8.0µmol/l]). In the following, pruritus progressed and dark colored urine appeared. Retesting at week 36 revealed markedly elevated serum bile acids (97.0µmol/l). Ursodeoxycholic acid (UDCA) treatment was initiated immediately. Four days prepartum, bilirubin increased (2.39mg/dl [1.0mg/dl]) and liver enzymes were elevated (GOT 51U/l [< 31U/l], GPT 56U/l [< 35U/l], AP 294U/l [35-104U/l]). During short-term UDCA treatment, bilirubin and GOT normalized, while GPT and AP decreased to 44U/l and 216U/l, respectively. C-section was carried out at 36 + 4 weeks. Two weeks postpartum, liver enzymes normalized, despite slightly elevated AP (123U/l). The patient had a history of recurrent gallstones and cholecystectomy 17 years before ICP and relatives with gallstones.

Whole Exome Sequencing and subsequent virtual panel analysis revealed a heterozygous in frame duplication p.(Arg32_Leu33dup) in the Semaphorin 7A (SEMA7A) gene. A homozygous missense variant was described by Pan et al. in PFIC. So far, no SEMA7A variant is known in ICP in literature. To elucidate the impact of the detected SEMA7A variant on ICP, the patient is included in the HiChol registry.

P2.27 Liver transplantation in a 64-year-old male patient with end stage liver disease due to langerhans cell histiocytosis

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Background Langerhans cell histiocytosis (LCH) is a rare neoplastic histiocytic disorder primarily affecting adults. In our case, a 64-year-old male underwent colonic polypectomy in another hospital, where unexpected histological findings confirmed LCH. This was later corroborated by a liver biopsy. Despite LCH diagnosis, treatment had not yet begun. Unfortunately, the patient developed secondary sclerosing cholangitis (SSC) and experienced rapid liver failure within six months. Consequently, he was transferred to our liver transplant center for potential transplantation.

Methods We assessed systemic Langerhans cell histiocytosis (LCH) manifestations using FDG-PET-CT imaging. Immunohistochemistry was employed to analyze CD1a, S100, and langerin expression in colonic, biliary, and explanted liver samples. Additionally, PCR analysis detected mutations in BRAF, KRAS, NRAS, and PIK3CA in these specimens.

Results The FDG-PET scan and histopathological analysis confirmed a single inactive LCH lesion in the cervical vertebra, treated with radiotherapy for stability. Liver transplantation was subsequently initiated due to progressive liver failure. The explanted liver showed biliary cirrhosis caused by secondary SSC with cholestasis, without active LCH. Immunosuppressive therapy with an mTOR inhibitor was initiated post-liver transplantation, and the patient is currently stable.

Conclusion LC) is an exceptionally rare disease, with hepatic involvement observed in approximately 10-15 % of cases. Complications arising from LCH may progress to secondary sclerosing cholangitis (SSC), a condition that can eventually culminate in end-stage liver disease necessitating liver transplantation. Globally, instances of LCH progressing to SSC and resulting in end-stage liver disease requiring liver transplantation in adults are exceedingly uncommon.

P2.28 Liver injury in malaria: from mild hepatitis to fulminant liver failure

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Background Endemic malaria was eradicated from Europe in the mid-20th century, however imported infections in non-endemic areas are now a growing concern. Liver damage is common in severe malaria but often overlooked in uncomplicated cases. Since 2012, we conducted comprehensive analyses of all malaria patients in our department.

Methods A retrospective, single-center study of patients with malaria was conducted at the Department of Internal Medicine I at the University Hospital Regensburg from January 2012 to June 2023, analyzing clinical, laboratory, microbiological, and epidemiological data from medical records.

Results 57 patients, mostly male (65 %), were analyzed. The majority (95 %) acquired malaria in sub-Saharan Africa. Approximately 32 % required intensive care and one patient died in our cohort. P. falciparum caused 96 % of cases. Thrombocytopenia was the main pathological laboratory finding. Noteworthy, about one-third of patients showed elevated liver enzymes. One critically ill patient experienced severe liver failure with coagulopathy (INR 7) and rare encephalitis with cytotoxic lesions of the corpus callosum. He eventually recovered after intensive care and was transferred to a regular ward within a month.

Conclusion Hepatopathy, while common in severe malaria, rarely leads to liver failure with coagulopathy. Our study shows that many malaria patients have elevated transaminase levels, suggesting liver damage. Although most cases are mild, some can escalate to severe liver failure, requiring intensive care. Consequently, we propose routine liver function assessment and enzyme testing for suspected or confirmed malaria cases.

P2.29 Metamizol assoziiertes akutes Leberversagen – Charakterisierung potentieller genetischer Marker

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Die medikamenteninduzierte Leberschädigung (DILI) ist ein heterogenes Erkrankungsbild mit einem breiten Spektrum an pathogenetischen Mechanismen. Für Metamizol sind bisher im Wesentlichen hämatologischen unerwünschte Arzneimittelwirkungen (UAWs), insbesondere eine Agranulozytose, beschrieben worden. Die hepatische Metabolisierung von Metamizol, seine Lipophilie und die relativ hohen Tagesdosis sind jedoch auch bekannte Risikofaktoren für eine DILI. Trotz seiner jahrzehntelangen Anwendung wurden bisher nur wenige Berichte über Leberschäden durch Metamizol veröffentlicht. In der Fachinformation wird auf eine mögliche Hepatitis und UAWs bei Leberzirrhose hingewiesen. Die Verschlechterung von Leberfunktionswerten oder ein akutes Leberversagen (ALF) werden nicht aufgeführt. Ziel: In einer Fallserie von bisher 5 PatientInnen mit reproduzierter (Reexposition), Metamizol-induzierter DILI erfolgte eine genetische Diagnostik (Whole-Exom-Sequenzierung (WES)) zur Charakterisierung potentielle genetischer Prädispositionen. Methoden: Die Diagnose von DILI basierte auf dem klinischen Verlauf (Metamizol Reexposition), dem histopathologischen Befund und dem RUCAM-Score. Die WES erfolgte in der hiesigen Humangenetik. Ergebnisse: Der klinische Verlauf der Fälle war charakterisiert durch die, in Unkenntnis der vorherigen Verläufe, mehrfache Reexposition von Metamizol im Rahmen von ärztlichen Therapien mit nachfolgendem ALF. Histopathologischen fand sich ein typisches DILI-Muster. Bei allen Patienten konnte eine Lebertransplantation vermieden werden. In der WES, die bisher eine genetische Mutation nachweisen konnte die mit einer Acyl-CoA-Dehydrogenase (MCAD)-Defizienz assoziiert ist. Zusätzlich zeigte sich eine Mutation die mit einer Dihydropyrimidin-Dehydrogenase-Defizienz assoziiert ist. Schlussfolgerung: In unserer Fallkohorte konnte erstmalig der Hinweis auf eine mögliche genetische Prädisposition für eine Metamizol DILI gezeigt werden. Diese Mutationen könnten in Zukunft als prognostischer Marker fungieren und somit schwere Verläufe einer iatrogenen, medikamenteninduzierten Leberschädigung bis hin zum Leberversagen durch Metamizol verhindern.

P2.30 Surgical versus Non-surgical Treatment for Hepatocellular Carcinoma with Macrovascular Invasion: A Systematic Review and Meta-analysis

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Background The prognosis of hepatocellular carcinoma (HCC) with macrovascular invasion (MaVI) is poor. The meta-analysis was aimed to compare the overall survival (OS) rates of surgical treatment (ST) with non-surgical treatment (NST).

Methods We searched studies from January 1990 to July 2023 through PubMed, Embase and Cochrane database. Primary and secondary subgroup analysis based on different types of tumor thrombosis were performed, including portal vein tumor thrombosis (PVTT) and hepatic vein tumor thrombosis (HVTT)/inferior vena cava tumor thrombosis (IVCTT).

Results A total of 33 studies were included. The 1-year, 3-year, and 5-year OS (All $P < 0.001$) were significantly higher in ST group. Primary subgroup analysis showed that ST group with PVTT displayed higher 1-year, 3-year and 5-year OS (All $P < 0.001$). Additionally, HCC patients with HVTT/IVCTT receiving ST had better 1-year ($P = 0.048$), 3-year ($P < 0.001$) and 5-year OS ($P = 0.046$). Secon-

dary subgroup analysis demonstrated that ST group with Type I PVTT exhibited longer 1-year OS (P = 0.001) while the difference was not observed in Type II PVTT. Surgery provided longer 3-year (P = 0.001; P < 0.001) and 5-year OS (P = 0.042; P = 0.001) for patients with Type I and II PVTT, respectively. The 1-year (P = 0.05), 3-year (P < 0.001) and 5-year (P = 0.027) OS of patients with HVTT were higher in ST group. There was no difference of OS in patients with IVCTT.

Conclusions Surgery should be recommended for HCC with Type I/II PVTT or HVTT.

P2.31 Transjugular Intrahepatic Portosystemic Shunt improves rebleeding and survival in gastric variceal hemorrhage

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Background and objective Variceal hemorrhage (VH) is a life-threatening complication of liver cirrhosis. Transjugular intrahepatic portosystemic shunt (TIPS) is so far the most efficient treatment for preventing rebleeding. TIPS also improves survival in selected patients populations with VH. However, the majority of studies conducted so far have focused on esophageal VH. The aim of this study is the assessment of the efficacy of TIPS in gastric VH.

Methods This study is a multicenter retrospective analysis of prospective data, including 429 patients with liver cirrhosis and VH. Our primary outcome was six-month mortality. The secondary outcome was occurrence of rebleeding within six months.

Results The median age was 58 years, most patients were males (72 %). 105 patients presented with gastric variceal hemorrhage (VH), of which 23 were treated with TIPS. 324 patients presented with esophageal VH, of which 72 were treated with TIPS. In gastric VH, TIPS treatment significantly improved 6-month survival (4.3 vs. 24.4 %, p = 0.034) and rebleeding rates (4.3 vs. 23.2 %, p = 0.042) compared to standard of care (SOC). Similary, TIPS treatment significantly improved 6-month survival (18.1 vs. 29.8 %, p = 0.049) and rebleeding rates (17.9 % vs. 6.9 %, p = 0.024) in esophageal VH as well.

Conclusion Secondary prophylaxis with TIPS significantly reduces the risk of rebleeding and improves six-month survival rates in both gastric and esophageal variceal hemorrhage (VH).

P2.32 Transjugular intrahepatic portosystemic shunt improves Quality of Life in patients with cirrhosis

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Background and Aims Health-related quality of life (HRQoL) in patients with decompensated liver cirrhosis is highly impaired. A transjugular intrahepatic portosystemic shunt (TIPS) is used to lessen portal pressure and the consequences thereof. This study aims to evaluate the influence of TIPS implantation on HRQoL in long-term follow-up in a multicenter cohort. Methods: 191 patients with liver cirrhosis from two centers were prospectively enrolled to evaluate HRQoL (MHH, 115; UKEssen, 76). All patients completed the Short Form Health Survey 36 (SF-36) at baseline before TIPS insertion and at structured early (1-3 months) and late follow-up (6-12 months after TIPS). Changes of ≥ 2.5 within the physical or mental component scale (PCS, MCS) were considered to be clinically important changes. Results: Predominant TIPS indication was ascites (70 %) and median MELD 12. Cirrhotic patients showed a reduced HRQoL compared to the German norm. 121 patients completed the SF-36 at early follow-up (~47 days after TIPS), 104 SF-36 were available at the later follow-up (~310 days after TIPS). HRQoL improved significantly between BL and early follow-up in PCS (p < 0.001), as well as PCS and MCS during late follow-up (both p < 0.001). Patients with ascites described inferior HRQoL at BL in comparison to individuals with variceal bleeding. Up to 70 % of ascites patients had a positive clinically important change of HRQoL at months 6-12.

Conclusion After TIPS insertion, HRQoL of patients with decompensated cirrhosis improved along short-term and long term follow-up. Especially ascites patients appeared to benefit from TIPS.

P2.33 Validation of Baveno VII criteria and other non-invasive diagnostic algorithms for clinically significant portal hypertension in hepatitis delta

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Background Limited data exists on the applicability of non-invasive tests (NIT) for clinically significant portal hypertension (CSPH) in chronic hepatitis delta (CHD).

Methods CHD patients with compensated advanced chronic liver disease (cACLD), defined by liver stiffness measurement (LSM) ≥ 10 kPa or advanced fibrosis/cirrhosison histology, who underwent paired assessment of hepatic venous pressure gradient (HVPG) and NIT at Hannover Medical School or Medical University of Vienna between 2013 and 2023 were retrospectively included. LSM, von Willebrand factor to platelet count ratio (VITRO), spleen stiffness measurement (SSM) were assessed, and combined linear CSPH risk models (ANTICIPATE, 3P/5P model) based on LSM and/or readily available laboratory biomarkers were assessed. The diagnostic accuracy of Baveno-VII criteria was investigated.

Results We included 51 CHD-cACLD patients with a CSPH prevalence of 62.7 %. The presence of CSPH was associated with higher LSM (25.8 [17.2-31.0] vs. 14.0 [10.5-19.8] kPa; p < 0.001), VITRO (n = 31, 3.5 [2.7-4.5] vs. 1.3 [0.6-2.0] %/[G/L]; p < 0.001), and SSM (n = 20, 53.8 [41.7-75.5] vs. 24.0 [17.0-33.9] kPa; p < 0.001). The AUROC for CSPH of linear risk models were excellent (ANTICIPATE: 0.885, 3P: 0.903, 5P: 0.912). The application of Baveno-VII criteria could rule out/rule in CSPH with 100 % sensitivity and 84.2 % specificity, respectively. The diagnostic 'grey zone' of Baveno-VII criteria was significantly diminished, whilst maintaining high accuracy, when applying the previously published Baveno-VII-VITRO or Baveno-VII-SSM algorithm.

Conclusions NIT for CSPH are useful and accurate in CHD-cACLD patients, and should be applied to identify patients at high risk for hepatic decompensation.

P2.34 Factors associated with the development of liver-related endpoints 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 for the development of lung and liver disease. Since the pace of liver disease progression and prognostic factors remain unknown, we evaluated host-related risk factors in the European Pi*ZZ liver cohort.

Methods 546 Pi*ZZ subjects without concomitant liver diseases, previous liver decompensation, or pathological alcohol consumption received a baseline clinical, laboratory, and elastographic assessment. 491 of them had a detailed follow-up interview at least six months after their baseline examination.

Results At baseline, 26 % and 13 % of Pi*ZZ individuals presented with a liver stiffness suggestive of significant and advanced fibrosis, respectively. 12 % had a BMI > 30kg/m2 and 3 % suffered under diabetes mellitus. During a median follow-up of 3.7 years, 26 individuals developed an hepatic endpoint (liver transplant/death, or decompensated cirrhosis). Pi*ZZ individuals with hepatic endpoint showed a significantly higher BMI (28 vs. 24 kg/m2, p = 2.7x10-4), were more often male (77 vs. 53 %, p = .018) and presented more often with diabetes mellitus (15 vs. 3 %, p = 7.9x10-4) at baseline, while no significant difference in alcohol consumption was noted. Cox regression analysis revealed diabetes mellitus (HR 5.7, 95 % CI 1.9-16.6, p = .002) and BMI > 30 kg/m2 (HR 4.4, 95 % CI 1.9-10.3, p = 6.8x10-4) as strong metabolic risk factors for liver-related endpoints. Male gender constituted a moderate risk factor (HR 3.0, 95 % CI 1.2-7.4, p = .021), while age > 50 years did not reach significance.

Conclusions In Pi*ZZ individuals, the presence of diabetes mellitus and BMI > 30 kg/m2 are strongly associated with the development of liver-related endpoints.

P2.35 Einfluss von Renin-Angiotensin-Inhibitoren auf den klinischen Verlauf sowie die Nierenfunktion von Patient*innen mit dekompensierter Leberzirrhose und Aszites

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Hintergund Studien haben gezeigt, dass die Inhibition des Renin-Angiotensin-Systems (RAS) in früheren Stadien der Lebererkrankung nephro- und hepatoprotektive Eigenschaften hat. Bei Patient*innen mit dekompensierter Zirrhose wird eine Erhöhung des Risikos für akutes Nierenversagen (AKI) vermutet, es liegen hierzu jedoch nur unzureichend Daten vor. Ziel dieser Studie war es, die Auswirkungen von RAS-Inhibitoren auf den klinischen Verlauf bei Personen mit dekompensierter Leberzirrhose zu untersuchen.

Methodik Insgesamt wurden 626 Patient*innen mit Leberzirrhose und Aszites eingeschlossen von denen 41 (7 %) Patient*innen einen RAS-Hemmer einnahmen. Endpunkte waren die Inzidenz von AKI und schwerem AKI (AKI Grad III) innerhalb von 28 Tagen und der langfristige Bedarf an Hämodialyse sowie das lebertransplantationsfreie (LTx-) Überleben. Patient*innen ohne RAS-Inhibitoren wurden mittels Propensity-Score-Matching im Verhältnis 3:1 mit Patient*innen mit RAS-Inhibitoren gematcht. Matching-Parameter waren MELD-Score, Alter, Thrombozyten, mittlerer arterieller Druck, Leukozyten und das Vorhandensein eines Typ-2-Diabetes mellitus.

Ergebnis Nach dem Matching wurden 117 Patient*innen ohne RAS-Inhibitoren mit 39 Patient*innen mit RAS-Inhibitoren verglichen. Innerhalb von 28 Tagen zeigten sich keine Unterschiede hinsichtlich der AKI Inzidenz (HR: 0,92, P = 0,81). Ein stratifizierter Log-Rank-Test zeigte eine signifikant niedrigere Inzidenz von schwerem AKI in der Gruppe mit RAS-Inhibitoren innerhalb von 28 Tagen (P < 0,001). Im 5-Jahres Verlauf war die Inzidenz von Hämodialyse bei Patient*innen mit RAS-Inhibitor-Einnahme verglichen zu Patient*innen ohne RAS-Inhibitoren signifikant geringer (HR: 0,22, P = 0,03). Das LTx-freie Überleben bei Patient*innen mit und ohne RAS-Inhibitoren war vergleichbar (HR: 0,83, P = 0,52).

Schlussfolgerung Die Einnahme von RAS-Hemmern bei Patient*innen mit dekompensierter Leberzirrhose ist mit einer geringeren Inzidenz von schwerem Nierenversagen und Hämodialysebedarf assoziiert.

P2.36 Implications of peak-MELD-scores for the outcome of patients listed for liver transplantation

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Introduction Since its introduction in 2002 the Model of End Stage Liver Disease (MELD)-score is used for liver allocation. As treatment options, donor age and patient characteristics change, the MELD-score needs regular revision. In fact, infections were identified as cause and consequence of acute decompensation. While leading to transient peaks of the MELD-score, infections also represent a main reason for changes in the waiting list status, thus hindering transplantation. Aim of this study was to investigate in detail the effect of peak-MELD-scores on waitlist outcome.

Methods In a retrospective study, patients newly registered for liver transplantation between 2020 and 2022 at the LMU Hospital were assessed for occurrence and consequences of a peak-MELD-score. This was defined as highest MELD-score within 6 months, increase in the MELD-score by 5 points or more within 3 months and subsequent decrease in the MELD-score. Calculations comprised the association of the peak-MELD-score with mortality, waiting time, waitlist status, infections and transplantation rate.

Results Of all patients 29 % deleloped a peak-MELD-score. These suffered from elevated mortality shortly after deleopment of the peak-MELD-score (Log-Rank Test; P = 0.03). Underlying was a low rate of transplantations (30,8 %), a high rate of infections developing alongside the peak-MELD-score (66.7 %) and a shorter time period in status "transplantable" thereafter (73,7 % of time vs. 86,9 %; P = 0.001).

Conclusion The occurrence of a peak-MELD-score marks a condition of elevated mortality and impaired access to liver transplantation, that requires more attention.

P2.37 TIPS insertion partially reverses systemic inflammation in patients with decompensated liver cirrhosis

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Background and Aims Patients with decompensated liver cirrhosis and portal hypertension are characterized by a state of systemic inflammation (SI). Transjugular intrahepatic portosystemic shunt (TIPS)-insertion is an effective thera-

py for portal hypertension. The aim of this study was to investigate the impact of TIPS-insertion on SI.

Methods 177 patients receiving a TIPS at Hannover Medical School were included. C-reactive protein (CRP) and white blood cells (WBC) were analyzed in 3-year-follow-up after TIPS-insertion. In a subset of 59 patients prospectively collected plasma samples were available for a comprehensive analysis of SI by measuring 48 soluble inflammatory markers (SIM) at baseline and 1, 3, 6 months after TIPS.

Results CRP levels, but not WBC, significantly decreased after TIPS-insertion ($p < 0.001$). Of note, CRP decline was associated with improved survival ($p = 0.030$). Pattern of SI was similar between patients with refractory ascites (RA) and variceal bleeding except for notably elevated IL-6 levels in patients with RA ($p < 0.001$). One month after TIPS, most SIMs remained at levels comparable to baseline with some, e.g. IL-2, even increasing. However, during further follow-up, we observed a continuous decline in most SIMs. At 6 months after TIPS 25 SIMs, including IL-6 and IL-2, showed significantly lower levels compared to baseline (FDR < 0.05).

While levels of 30/48 SIMs were significantly higher in the patients' blood compared to healthy controls at baseline, 7 of these had reversed to healthy control-levels after 6 months.

Conclusions Decreasing portal hypertension via TIPS-insertion leads to significant improvement of SI over time.

P2.38 Cause and severity of patients referred to a university specialty outpatient clinic for unclear hepatopathy

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Background Elevated liver enzymes are a frequent finding in the German population. Misdiagnosis of the underlying liver disease may enhance progressive liver impairment, but data regarding the etiology and extent of fibrosis are lacking. In 2019, we established a specialized outpatient clinic where primary care physicians can refer patients with unclear hepatopathy.

Methods Retrospective analysis of all patients presenting to the outpatient clinic between 01. January and 31 December 2019. All patients received serologic testing for viral and autoimmune liver disease, for M. Wilson, hemochromatosis and alpha 1 deficiency. Abdominal ultrasound and liver stiffness measurement (LSM; Fibrocan) completed diagnostic workup.

Results A total of n = 597 patients were analyzed. Nonalcoholic fatty liver disease (NAFLD, n = 237); alcoholic liver disease (ALD, n = 51), primary biliary cholangitis (PBC, n = 26), and autoimmune hepatitis (AIH, n = 21) were the most prevalent diseases. According to fibroscan moderate fibrosis (F; F ≥ 2 °) was found in NALFD n = 25 (10 %), ALD n = 16 (31 %), PBC n = 5 (19 %), AIH n = 7 (33 %). Severe fibrosis was present in: NAFLD n = 37 (16 %); ALD n = 2(4 %); PBC n = 2 (9 %) and AIH n = 3 (14 %)).

Conclusion In this cohort study, NAFLD was the most prevalent liver disease and was associated with the highest rate of consecutive liver fibrosis. Furthermore, proportion of severe fibrosis was highest in patients with initial diagnosis of autoimmune liver disease (AIH, PBC). Since NAFLD is a preventable disease and autoimmune liver disease requires medical treatment to prevent disease progression, patients with unexplained hepatopathy should timely receive a structured diagnostic workup.

P2.39 Are minimal hepatic encephalopathy tests comparable regarding their predictive value and diagnostic accuracy?

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Introduction Minimal hepatic encephalopathy (mHE) is associated with poor outcome in cirrhotic patients. There is limited information regarding the concordance of various diagnostic mHE tests and their ability to predict clinical outcome. In this prospective study, we evaluated six mHE tests and their predictive potential concerning overt hepatic encephalopathy (oHE), rehospitalization, and mortality.

Methods mHE assessment consisted of psychometric hepatic encephalopathy score (PHES), Animal Naming Test (ANT), Critical Flicker Frequency (CFF), Inhibitory Control Test (ICT), EncephalApp (Stroop) and Continuous Reaction Time Test (CRT). We performed time-dependent analyses to determine the predictive value of these tests for oHE development, rehospitalization and mortality during 365 days follow-up.

Results Of 132 analysed patients, 23 (17.4 %) presented with HE grade 1-2 at baseline. Regarding the 109 (82.6 %) neurologically unimpaired patients, mHE was detected in 35.8 % (PHES), 44.0 % (CRT), 52.3 % (Stroop), 51.4 % (ANT), 36.7 % (ICT) and 25.7 % (CFF) of patients. Patients with HE grades 1-2 achieved abnormal test results in 100 % (PHES), 82.6 % (CRT), 95.7 % (Stroop), 91.3 % (ANT), 78.3 % (ICT), and 39.1 % (CFF). During 365 days follow up, 24 (18 %) patients developed oHE, 58 (44 %) were re-admitted to hospital and 20 (15.2 %) died. In multivariable model, PHES and ANT were associated with oHE development. No other test was linked to clinical outcome.

Conclusion Frequency of abnormal tests results varied significantly between the different evaluated tests. This demonstrates that the different mHE tests cannot be equated with each other. PHES and ANT seem to be most useful for identifying those with high risk for oHE development.

P2.40 Kv7-channel blockage reduces liver ischemia-reperfusion injury in mice

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Background and Objectives Liver ischemia-reperfusion injury (IRI) occurs during various liver surgeries and transplantations due to disruption of blood flow. IRI can cause substantial liver damage and inflammation, which can lead to liver or transplant failure. Kv7 channels, voltage-gated, cyclic nucleotide-gated potassium channels, which are important for vascular smooth muscle cell regulation, have been studied in the context of IRI specifically in the heart. The aim of this study is to investigate the involvement of Kv7 potassium channels in mouse liver IRI.

Methods A temporary clamp on the left lobe of the liver induced partial liver IRI for 60 minutes after application of Kv7 channel modulators Retigabine (n = 30), XE991 (n = 30), and Linopiridine (n = 30) in C57BL/6-mice. Liver injury was analyzed using serum markers AST and ALT, histological studies with H.E. staining. Further analysis included immunohistochemical staining for cleaved caspase-3 and myeloperoxidase (MPO). Pathway analysis was performed using Western blot analysis.

Results Mice treated with Kv7-channel blockers showed significant reductions in AST and ALT levels. Histopathological analysis revealed that mice treated with Kv7-channel antagonists experienced significantly less necrosis after 24

hours of reperfusion compared to controls. Further research showed that there was less inflammation and liver injury in the treated group.

Conclusion Blocking hepatic Kv7 channels effectively reduces liver IRI in mice. These results suggest that Kv7 modulators may represent a new therapeutic option for reducing IRI and even prevent liver injury and failure after liver surgery or transplantation.

P2.41 The outcome after liver transplantation may be affected by the circadian rhythm of liver transplant donors

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Introduction The human circadian clocks' central regulator is located in the suprachiasmatic nucleus in the hypothalamus regulating local circadian clocks in peripheral tissues. It influences rhythmic physiology such as detoxification, metabolism, hormonal secretion, and nutrient uptake. Impaired hepatic circadian clocks are associated with metabolic and inflammatory liver diseases. In this study, we therefore aimed to investigate associations of the circadian phase of liver transplant donation with the outcome of patients after liver transplantation.

Methods Clinical data were analyzed of 417 patients who had received a liver transplantation at the LMU University Hospital since 2014. Circadian times were defined as night (8 p.m. – 8 a.m.) or day time (8:01 a.m. – 7:59 p.m.). Associations between circadian time of donor liver harvesting and outcome of patients (survival, graft loss) was analyzed. Circadian clock genes were investigated by RT-PCR.

Results Patients who received donor livers that were harvested during circadian day time showed a trend to a lower survival within 90 days compared to patients who received a liver that was harvested during circadian night time (86.3 % vs. 90.9 %, P = 0.07). Of note, the circadian time of liver transplantation had no impact on the outcome of patients (P = 0.9), making it unlikely that the performance of the medical teams during varying circadian times is responsible for the observed differences.

Conclusion According to this preliminary analysis, the circadian clock state of the donor liver may affect the recipients' survival after liver transplantation. Results of circadian clock gene expression will be presented.

P2.42 The wish to give- increasing the living liver donor rate by optimal interdisciplinary donor managemant. An experience from a single center in the Middle East

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In the Middle East there is a high demand for living donors due to the lack of deceased organ donors. KFSHRC started in 2010 with open surgery for living liver donation and an in-hospital stay of apporoximately 7 days. In 2015 laparoscopic donor surgery by was initiated, donor workup was expedited and completed within 3 weeks and in-hospital stay was reduced to 5-6 days. However the laparoscopic approach was not satisfactory, so robotic donor surgery was implimented reducing in-hospital stay to 3-6 days for either left latera,l full left or right lobe donation. Up now more than 700 robotic donor hepatectomies were performed in a single centre. Donors were supported by hepatologists, psychologists, social workers and nurses. Optimal schedules were tailored around the donors need with regards to compensation for salary, sick leave and re-commencing of work. Donors were able to restart work after 2-4 weeks depending on the type of work. Travelling was able after 4-6 weeks for work or leisure. When these results were noticed amongst patients and families of patients with an indictaion for liver transplantation the wish to donate was expressed by many more volunteering donors. Eventually optimal donor management gave us the opportunity to expand living donation to unrelated direct and even indirect living liver donation with excellent results. Although population characteristics and family structures in the West are not comparable with the Middle East, much higher rates of living liver donation could be realized in wetsern countires by optimizing donor management.

P2.43 Circulating miRNA-21-5p as a Biomarker for Predicting Clinical Outcomes in Decompensated Liver Cirrhosis: Insights from Large Patient Cohort

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Chronic liver diseases, particularly cirrhosis, impose a substantial healthcare burden globally, characterized by high morbidity and mortality rates. Decompensated cirrhosis frequently progresses to acute-on-chronic liver failure (ACLF), marked by multi-organ dysfunction and elevated 28-day mortality. Accurate prediction of ACLF progression is critical for optimizing patient care. This study focuses on the potential of circulating microRNAs (miRNAs) as biomarkers for advanced liver disease, with a specific emphasis on ACLF.

First, in a pilot study representative patient groups, including ACLF, decompensated cirrhosis, and compensated cirrhosis, miRNA sequencing unveiled notable variations between ACLF patients and controls. Especially, miRNA-21-5p exhibited a substantial increase in ACLF patients. The validation process was carried out in two extensive patient cohorts, namely, ACLARA (n = 522) and PREDICT (n = 722). miRNA-21-5p significantly correlated with markers of liver function in these large patient cohorts. Furthermore, high serum levels of miRNA-21-5p were associated with unfavorable clinical outcomes, including decreased 90-day survival.

These findings collectively suggest that miRNA-21-5p has the potential to serve as a reliable predictor of clinical outcomes and resource utilization in patients with decompensated liver cirrhosis. While these results hold promise, additional research is warranted to gain a more comprehensive understanding of the molecular mechanisms and clinical implications linked to altered miRNA-21-5p serum levels.

P2.44 Safety of coronary angiography in patients with cirrhosis as part of an evaluation for liver transplantation

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Coronary angiography (CA) is often performed as part of the evaluation for liver transplantation (LT) and may include stent placement through percutaneous coronary intervention (PCI) followed by dual antiplatelet therapy (DAPT). However, data on the impact of PCI with DAPT in patients with cirrhosis is limited and data on the induction of acute-on-chronic liver failure (ACLF) are not available. We therefore aimed to assess the safety of CA with or without stenting regarding bleeding events, development of ACLF and survival. Clinical data of patients with cirrhosis who underwent CA as part of an evaluation for LT at the

University Hospital Essen between 2008 and 2022 were retrospectively collected and statistically analysed. Associations with bleedings, ACLF and transplant-free survival were assessed in regression models. A total of 318 patients with cirrhosis who underwent CA were included. PCI with subsequent DAPT was performed in 46 cases (14 %). Bleeding events in the first six months after CA were seen in 34 cases (11 %) (stent group n = 10, 22 %; no-stent group n = 24, 9 %, p = 0.022). Stenting was significantly associated with bleeding events (OR = 3.44, 95 % CI [1.14, 10.29], p = 0.028) and ACLF (OR = 5.80, 95 % CI [1.70, 19.71], p = 0.005). Competing risk survival analysis revealed that stenting was significantly associated with worse transplant-free survival (sHR = 1.92, 95 % CI [1.13,3.27], p = 0.015). Our data show that PCI with subsequent DAPT in patients with cirrhosis is associated with an increased risk of bleeding events, development of ACLF and worse transplant-free survival. Although CA remains an important part of the evaluation process for LT.

P2.45 Body composition is associated with postoperative complications in perihilar cholangiocarcinoma

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Perihilar cholangiocarcinoma (pCCA) is a malignant tumor of the hepatobiliary system associated with a challenging prognosis. Changes in body composition (BC) impact the prognosis of various tumors. Our study aimed to investigate the correlation between BC, postoperative complications and oncological outcome in patients with pCCA. All patients with pCCA who underwent curative-intent surgery between 2010 and 2022 in a single hepatobiliary center were included. BC was assessed using preoperative computed tomography and analyzed with the assistance of a 3D Slicer software. Binary logistic regression analyses were conducted to examine the relationship between BC and clinical characteristics including postoperative complications and Cox regressions and Kaplan-Meier analysis to evaluate oncological risk factors. BC was frequently altered in patients undergoing liver resection for pCCA (n = 204) with 52.5 % of the patients showing obesity, 55.9 % sarcopenia, 21.6 % sarcopenic obesity, 48.5 % myosteatosis and 69.1 % visceral obesity. In multivariate analysis, postoperative complications (Clavien-Dindo ≥ 3b) were associated with body mass index (Odds ratio (OR) = 2.001, p = 0.024), sarcopenia (OR = 2.145, p = 0.034), and myosteatosis (OR = 2.097, p = 0.017) as independent predictors. Sarcopenia was associated with reduced overall survival in pCCA patients (sarcopenia vs. no-sarcopenia, 21 months vs 32 months, p = 0.048 log rank). Altered BC is major problem in pCCA patients. More than half of them show notable changes in BC due to cancer-cachexia and chronic infections. In our large cohort, we demonstrated an association of altered BC with severe postoperative complications in patients who underwent liver resection and show a tendency of impaired overall survival. Preoperative assessment and improvement of BC might improve outcomes.

P2.46 Prediction of long-term survival after surgical resection for perihilar cholangiocarcinoma

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Perihilar Cholangiocarcinoma (pCCA) remains a challenging liver malignancy due to its high perioperative complications and dismal long-term prognosis with only a few long-term survivors (LTS). We investigated characteristics and predictors of LTS in pCCA patients. Patients undergoing curative-intent liver resection for pCCA between 2010 and 2022 were categorized into long-term and short-term survivors (STS) excluding perioperative mortality. Binary logistic regression was used to determine key differences between the groups and to develop a survival predictor. This predictor variable was tested in the whole cohort of surgically treated pCCA patients using Cox Regression analysis for cancer-specific survival (CCS).Within a cohort of 209 individuals, 27 patients were identified as LTS (median CCS = 125 months) and 55 patients as STS (CCS = 16 months). Multivariable analysis identified preoperative portal vein filtration (OR = 5.85, p = 0.018) and intraoperative packed red blood cell (PRBC) transfusions (OR = 10.29, p = 0.002) as key differences between the groups. A predictor variable based on these two features was created and transferred into a Cox regression model. Here, the predictor variable (HR = 0.35, p < 0.001) and nodal metastases (HR = 2.15, p = 0.001) as well as postoperative complications (HR = 3.06, p < 0.001) were identified as independently associated with CCS. Long-term survival after surgery for pCCA is possible and was strongly associated with preoperative portal vein filtration and intraoperative PRBC transfusion. As both variables are part of preoperative staging or can be modulated by intraoperative technique, the proposed predictor variable can easily be transferred into clinical management to predict the oncological risk of pCCA patients undergoing surgery.

P2.47 Body composition in cholangiocarcinoma affects immune cell populations in the tumor and normal liver parenchyma

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Due to malnutrition and tumor cachexia, body composition (BC) is frequently altered and known to adversely affect short- and long-term results in patients with cholangiocarcinoma (CCA). Here, we explored immune cell populations in the tumor and liver of CCA patients with respect to BC. A cohort of 96 patients who underwent surgery for CCA was investigated by multiplexed immunofluorescence (MIF) techniques with computer-based analysis on whole tissue slide scans to quan-tify and characterize immune cells in normal liver and tumor regions. BC was characterized by obesity, sarcopenia, myosteatosis, visceral obesity and sarcopenic obesity. Associations between BC and immune cell populations were determined by univariate and multivariable binary lo-gistic regressions. BC was frequently altered in intrahepatic CCA (iCCA, n = 48), with 47.9 % of the patients showing obesity, 70.8 % sarcopenia, 18.8 % sarcopenic obesity, 58.3 % myosteatosis and 54.2 % visceral obesity as well as in perihilar CCA (pCCA, n = 48) with 45.8 % of the patients showing obesity, 54.0 sarcopenia, 14.6 % sarcopenic obesity, 47.9 % myosteatosis and 56.3 % vis-ceral obesity. From an immune cell perspective, independent associations within the tumor com-partment were observed for myosteatosis (iCCA: TIM-3 + CD8 + cells; pCCA: PD-L2 + CD68-cells, CD4 + cells) and obesity (iCCA: PD-1 + TIM-3 + CD4 + cells) and within the normal liver parenchyma for sarcopenia (pCCA: CD68 + cells, TIM-3 + CD8 + cells),visceral obesity (iCCA: PD-1 + PD-L1 + PD-L2 + CD68 + cells; pCCA: ICOS + -TIGIT + CD8 + cells) and sarcopenic obesity (pCCA: PD-1 + PD-L1 + PD-L2 + CD8 + cells). This is the first systematic analysis of the association of BC and immune cells in cholangiocarcinoma showing a strong association between BC and distinct im-mune cell populations within the tumor itself as well as within the normal parenchyma.

P2.48 Multiparametric ultrasound-based assessment of liver grafts for the detection of graft fibrosis and steatosis

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Introduction Patients after liver transplantation require close monitoring for graft steatosis and fibrosis due to the risk for metabolic complications from immunosuppressants or liver disease recurrence. Transient elastography (TE) was shown to be able to detect graft fibrosis, whereas controlled attenuation parameter (CAP) could identify graft steatosis. This study aimed to evaluate the effectiveness of three methods for non-invasive multiparametric assessment of graft fibrosis and steatosis in liver transplant recipients.

Methods 38 patients were included in this pilot study. Graft fibrosis and steatosis were assessed through ultrasound-based techniques embedded on the Hologic MACH 30 machine: ShearWave PLUS Elastography (SWPE), Attenuation PLUS (AttPLUS) and Sound Speed PLUS (SSP). Non-invasive serum-based tests for fibrosis were calculated. Transient Elastography (TE) and Controlled Attenuation Parameter (CAP) performed on a FibroScan 630 Expert were considered as reference.

Results 44.7 % of our cohort were female with a median time since transplantation of 3.58 years. 7 patients showed fibrosis on TE. SWPE showed the best predictive value for liver fibrosis with an AUROC of 0.922. NITs performed poor with AUROCs for FIB-4, APRI and NFS of 0.615, 0.622 and 0.577, respectively. SWPE and liver stiffness measurement by TE correlated significantly ($r = 0.589$, $p < 0.001$). For steatosis assessment, SSP measurements showed a significant inverse correlation with CAP ($r = -0.605$, $p < 0.001$), whereas AttPLUS did not correlate with CAP values.

Conclusion Multiparametric ultrasound assessment showed promising results in assessing graft fibrosis and steatosis. However, larger studies are required to validate these findings and to identify clinically significant cut-offs.

P2.49 Partial liver resection alters the bile salt-FGF19 axis in patients with perihilar cholangiocarcinoma: implications for liver regeneration

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Background & Aims Extended liver resection is the only treatment option for perihilar cholangiocarcinoma (pCCA). Bile salts and the gut hormone FGF19, both promoters of liver regeneration (LR), have not been investigated in patients undergoing resection for pCCA. We aimed to study the bile salt-FGF19 axis perioperatively in pCCA and study its effects on LR.

Methods Plasma bile salts, FGF19, and C4 (bile salt synthesis marker) were assessed in patients with pCCA and controls (colorectal liver metastases) before and after resection on postoperative days (POD) 1, 3 and 7. Hepatic bile salts were studied using intraoperative liver biopsies.

Results Partial liver resection in pCCA elicited a sharp decline in bile salt and FGF19 plasma levels on POD 1 and remained low thereafter, unlike in controls, where bile salts rose gradually. Preoperatively, suppressed C4 in pCCA normalized postoperatively to levels similar to those in the controls. The remnant liver volume and postoperative bilirubin levels were negatively associated with postoperative C4 levels. Furthermore, patients who developed postoperative liver failure had nearly undetectable C4 levels on POD 7. Hepatic bile salts strongly predicted hyperbilirubinemia on POD 7 in both groups. Finally, systemic bile salts and FGF19 were not associated with LR in patients with pCCA, as opposed to a positive association between postoperative bile salts and LR in controls.

Conclusions Partial liver resection alters the bile salt-FGF19 axis, and its derailment is unrelated to LR in pCCA. Postoperative monitoring of circulating bile salts and their production may be useful for monitoring LR.

P2.50 Results of the prospective international multicenter DRAGON 1 trial investigating combined portal and hepatic vein embolization in patients with colorectal liver metastases and small future liver remnants

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Introduction Patients with extensive colorectal liver metastases (CRLM) often require Future Liver Remnant (FLR) hypertrophy-inducing procedures to minimize the risk of developing post-hepatectomy liver failure. Combined Portal and Hepatic Vein Embolization (PVE/HVE) may accelerate FLR hypertrophy and lead to higher resection rates. The aim of the DRAGON 1 trial is to assess the training, implementation, feasibility, safety, and efficacy of PVE/HVE in patients with primarily unresectable CRLM.

Methods The DRAGON 1 is a prospective, single-arm, international, multicenter trial. The primary endpoint is the ability of each center to recruit 3 patients within 12 months, without 90-day mortality due to complications related to the PVE/HVE procedure. Secondary endpoints include resection rate, FLR-hypertrophy, complications, 90-day mortality post-resection, recurrence and 1-year survival

Results A total of 102 patients from 43 centers across 14 countries were enrolled between May 2020 and October 2022. 24 of the 43 centers were able to enroll 3 patients. No procedure-related mortality was reported. There was one serious adverse event related to the intervention. PVE/HVE resulted in a high kinetic growth rate and 90 % of the patients were resected.

Conclusions The DRAGON 1 trial demonstrates that PVE/HVE is safe and 56 % of the participating centers were able to recruit 3 patients. Additionally, a high kinetic growth rate resulted in a resection rate of 90 % after PVE/HVE.

P2.51 What makes a Difficult Liver Transplantation?

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Introduction Although liver transplantation (LT) is among the most complicated abdominal surgical procedures, surgical difficulty of LT was to date not objectively defined. Azoulay proposed recently a point-based system, depending on cold ischemia time (CIT), blood units transfused intraoperatively, and duration of operation, which showed promising results in short-term outcomes in two centers in France and Spain. Our aim was to validate this model in our high-output LT center in the Eurotransplant region and to combine donor and recipient data to highlight high-risk combinations to aid transplant physicians when considering individual candidates.

Methods We retrospectively analyzed 781 patients who were transplanted between January 2010 and January 2023 at our center. Points were assigned as by Azoulay with a modification, as instead of CIT, duration of hepatectomy was calculated. All values at or above the median value of the whole population defined a difficult LT. The correlation of difficult LT with 90-day mortality was

assessed. Predictors of difficult LT will be identified via multivariable analysis using recipient, donor, and surgical data.

Results The incidence of difficult LT was 19.3 % in our cohort. Difficult LT was associated with a lower 90-day survival (71.5 % vs. 88.5 % in case of non-difficult LT, p < 0.0001). Predictors of a difficult LT will be assessed by multivariable analysis.

Discussion Difficult LT as defined here occurs frequently and results in poorer postoperative outcomes. Early recognition of these cases, adjusting risk factors and optimizing donor-recipient matching may improve future results.

P2.52 Surgical resection of an initially unresectable hepatocellular carcinoma after combined systemic and local neoadjuvant therapy – a case report

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Introduction Although neoadjuvant therapy is widely used in the management of several malignant tumors, it is not yet an established therapeutic concept for patients with locally advanced, unresectable hepatocellular carcinoma.

Case report In May 2023, a 29-year-old patient presented with the primary diagnosis of large hepatocellular carcinoma in liver segments VII/IV/V/VI (14x12x14 cm) and a thrombosis of the superior mesenteric vein. He had a history of hepatitis B without detectable HBV DNA and a normal liver function assessed by LiMAX. The highest AFP value was 2.217.700 µg/l. Esophageal varices grade II-III with red spots were treated with endoscopic band ligation. After interdisciplinary discussion in our liver board, we employed an individualized therapeutic regimen consisting of TACE and immunotherapy, with the intention to enable resection after tumor downsizing. Due to thrombosis of the superior mesenteric vein and presence of esophageal varices an anti-VGEF-free regimen with Durvalumab/Tremelimumab was started in May 2023. In addition, two transarterial chemoemoblizations were performed (06/2023 and 08/2023). With this therapeutic approach the tumor size significantly decreased to 10x8x4 cm with only minimal tumor vitality in the MRI, also indicated by the significant drop of AFP to 138 µg/l. On October 25th 2023 we performed a central liver resection of the segments IVb/V. The intraoperative histological quick section confirmed a microscopically-negative surgical margin.

Conclusion Here we present an impressive tumor downsizing under combined systemic and local therapy, resulting in a successful liver resection in a tumor situation, which would be classified as palliative before the advent of immunotherapy.

P2.53 Preoperative predictors for non-resectability in perihilar cholangiocarcinoma

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Introduction Explorative laparotomy without subsequent curative-intent liver resection remains a major clinical problem in the treatment of perihiliar cholangiocarcinoma (pCCA). Thus, we aimed to identify preoperative risk factors for non-resectability of pCCA patients.

Material and Methods Patients undergoing surgical exploration between 2010 and 2022 were eligible for our analysis. Separate binary logistic regressions analyses were used to determine risk factors for non-resectablity after explorative laparotomy due to technical (tumor extent, vessel infiltration) and oncological (peritoneal carcinomatosis, distant nodal or liver metastases)/liver function reasons.

Results Our monocentric cohort comprised 318 patients with 209 (65.7 %) being surgically resected and 109 (34.3 %) being surgically explored (explorative laparotomy: 87 (27.4 %), laparoscopic exploration: 22 (6.9 %)). Risk factors for non-resectablity after explorative laparotomy were advanced age (HR = 3.76, p = 0.003), portal vein embolization (PVE, HR = 5.73, p:0.007) and arterial infiltration > 180 ° (HR = 8.05 p < 0.001) for technical non-resectablity and PVE (HR = 4.67, p = 0.018), arterial infiltration > 180 ° (HR = 3.24, p = 0.015) and elevated CA 19-9 (HR = 3.2, p = 0.009) for oncological non-resectablility.

Conclusion Advanced age, PVE, arterial infiltration and elevated CA 19-9 are major risk factor for non-resectablity in pCCA. Diagnostic laparoscopy, especially in high-risk situations, should be used to reduce the amount of explorative laparotomies without subsequent liver resection.

P2.54 Impact of altered body composition on clinical and oncological outcome in intrahepatic cholangiocarcinoma

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Introduction Intrahepatic cholangiocarcinoma is a common primary liver tumor with limited treatment op-tions and poor prognosis. Changes in body composition (BC) have been shown to affect the prog-nosis of various types of tumors. Therefore, our study aimed to investigate the correlation be-tween BC and clinical and oncological outcomes in patients with iCCA.

Methods All patients with iCCA who had surgery from 2010 to 2022 at our institution were included. We used CT scans and 3D Slicer software to assess BC and conducted logistic regressions as well as cox regressions and Kaplan-Meier analysis to investigate associations of BC to clinical variables with focus on post-operative complications and oncological outcome.

Results BC was frequently altered in iCCA (n = 162) with 53.1 % of the patients showing obesity, 63.2 % sarcopenia, 52.8 % myosteatosis ,10.1 % viscer-al obesity and 15.3 % sarcopenic obesity. Multivariate analysis showed no meaningful associa-tion between BC and perioperative complications. Myosteatosis was associated with reduced overall survival (OS) in iCCA patients (myosteatosis vs. non-myosteatosis, 7 vs. 18 months, p = 0.016 log rank). Further, subgroup analysis revealed a notable effect in the subset of R0 resected patients (myosteatosis vs. non-myosteatosis, 18 vs. 32 months, p = 0.025) and patients with nodal metastases (myosteatosis vs. non-myosteatosis, 7 vs. 18 months, p = 0.016).

Conclusion While altered BC is not associated with perioperative outcomes in iCCA, myosteatosis emerges as a prognostic factor for reduced OS in the overall and sub-populations of resected patients.

P2.55 Combining clinical data and systemic inflammation to predict the outcome after TIPS: An AI-based approach

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Background Scores like the Freiburg-index of post-TIPS survival (FIPS) and the CLIF-Consortium Acute Decompensation score (CLIF-C ADs), mainly focusing on limited clinical data, have recently been proposed to predict clinical outcome after transjugular-intrahepatic-portosystemic-shunt (TIPS) insertion. Our study aimed to demonstrate machine learning potential in combining various information about systemic inflammation and clinical data to predict post-TIPS outcome.

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Methods We used a dataset of 64 patients with 60 features (17 baseline parameters and 43 cytokines) to predict the combined endpoint of acute-on-chronic-liver-failure, liver-transplantation, and death during one-year follow-up. Predominant TIPS indication was refractory ascites (75 %, n = 48). Model evaluation employed weighted F1 scores, Youden index, and five-fold cross-validation. The machine learning approach included data scaling, feature selection, and evaluation of various algorithms (Logistic Regression, Support Vector Machine, Random Forests, Gradient Boosted Classification) on an 80/20 training-testing split.

Results The combined endpoint occurred in 17 patients (27 %). The Logistic Regression Classifier appeared as the top performer with a weighted F1 score of 0.79 and an ROC AUC of 0.95 [95 % CI: 0.88 – 1.00]. Of note, the model was superior when compared to FIPS ($p < 0.001$), CLIF-C ADs ($p < 0.001$), and MELD score ($p < 0.001$), that yielded ROC AUC values of 0.70 [95 % CI: 0.56 – 0.83], 0.43 [95 % CI: 0.27 – 0.59], and 0.43 [95 % CI: 0.28 – 0.59], respectively.

Conclusion Machine learning offers a promising approach for improved prediction of TIPS outcomes, outperforming established scores in our cohort. However, validation of our model is still pending.

P2.56 Vergleich von getunnelten Peritonealkathetern und Alfapump bei Patient:innen mit Leberzirrhose und therapierefraktärem Aszites

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DOI 10.1055/s-0043-1777560

Einleitung Die aktuelle Standardbehandlung von Patient:innen mit therapierefraktärem Aszites sind großvolumige Parazentesen, sofern eine Lebertransplantation (LTx) oder die Anlage eines transjugulären-intrahepatischen Shunts nicht möglich ist. Neue Behandlungsmöglichkeiten wie getunnelte Peritonealkatheter (PK) oder die Alfapump bieten Alternativen für die Aszitestherapie in der Häuslichkeit. Einen Vergleich beider Devices hinsichtlich wichtiger klinischer Endpunkte gibt es zurzeit nicht. Diese Studie vergleicht den klinischen Verlauf von Patient:innen mit Alfapump und PK.

Methoden In dieser Studie wurden alle Patient:innen mit Leberzirrhose eingeschlossen, die von 2012-2023 in der Medizinischen Hochschule Hannover einen PK oder eine Alfapump erhielten. Untersuchte Endpunkte im Ein-Jahresfollow-up waren LTx-freies Überleben, akutes Nierenversagen (AKI), Hyponatriämie, sowie Deviceexplantation. Time-to-event Analysen wurden mittels multivariabler Cox-Regression, adjustiert für FIPS-Score und täglicher Drainagemenge, untersucht.

Ergebnisse Insgesamt wurden 177 Patient:innen mit PK und 26 mit Alfapump betrachtet. Patient:innen mit PK hatten einen höheren FIPS-Score (0,7 ± 0,9 vs 0,2 ± 0,9, p = 0.016) und waren älter (61 ± 11 vs 55 ± 12, p = 0.009). Im ein Jahres follow-up fand sich kein signifikanter Unterschied im Auftreten von AKI (PK: 87,0 %, Alfapump: 95,2 %, HR 1,172, KI 0,740-1,856, p = 0.499), Hyponatriämie (PK: 80,0 %, Alfapump: 86,7 %, HR 0,794, KI 0,472-1,334, p = 0.383) und LTx-freies Überleben (PK: 40,2 %, Alfapump: 65,7 %, HR 0,607, KI 0,268-1,373, p = 0.230). PK-Patient:innen wiesen eine höhere Explantationsrate innerhalb eines Jahres auf (PK: 87,4 %, Alfapump: 55,3 %, HR: 0,357, p = 0.004, mediane Zeit zur Explantation 30 Tage vs. 53 Tage).

Schlussfolgerung Das Auftreten klinischer Komplikationen von Patient:Innen mit Devices und refraktärem Aszites ist unabhängig von der Deviceart häufig. Dennoch ist die Liegedauer der Alfapump länger als die eines PK.

Poster Visit Session III METABOLISM (INCL. MASLD) 26/01/2024, 16.25pm – 17.00pm

P3.01 Metallothionein immunohistochemistry is a highly sensitive biomarker for Wilson Disease

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Wilson disease (WD) is caused by mutations in the atp7b gene resulting in copper accumulation in liver, brain, and cornea. Hepatic involvement may be asymptomatic until cirrhosis or may manifest as fulminant hepatic failure. The diagnosis can be difficult, as both clinical criteria and genetic testing have limitations. Histochemical staining of copper and copper-associated protein is used during evaluation of liver biopsies with suspected WD. Here, the diagnostic performance of metallothionein (MT) was evaluated.

MT immunohistochemistry was performed on liver specimens of WD patients (n = 64) and controls (n = 160). The cut-off for detection of WD was determined by ROC analysis.

An at least moderate staining in > 50 % of hepatocytes was observed in 56 of 69 liver samples from WD patients, while only five control cases showed this staining pattern. The sensitivity, specificity, and accuracy for new diagnosis of WD were 86 %, 97 %, and 95 %, respectively. Importantly, sensitivity in non-fibrotic patients was 72 %, a diagnostic setting in which histochemistry failed (rhodanine 11 %, orcein 0 %). Patients with MT-negative liver samples showed less urinary copper excretion compared to MT-positive cases (median 0.77 vs. 2.62 µmol/d, $p < 0.05$), while hepatic copper concentration was similar between both subgroups. The substitution of hepatic copper concentration by MT immunohistochemistry resulted in a modified Leipzig score ≥ 4 in 97 % of WD patients and 95 % of WD patients were correctly classified after replacing atp7b genotyping by MT immunohistochemistry.

Thus, MT is an excellent biomarker for histological diagnosis of WD and replace rhodanine in a modified Leipzig score.

P3.02 Characterising MASLD using Bayesian Networks in the UK Biobank

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DOI 10.1055/s-0043-1777562

Background Quick and accessible methods that characterise and predict Metabolic dysfunction-associated steatotic liver disease (MASLD) remain paramount considering its increasing prevalence. A continued area of focus is the use of blood biomarkers to estimate liver steatosis. We present a Bayesian Network approach which describes the structure of MASLD, using 35,000 UK Biobank participants.

Methods Using a Random Forest Classifier (RFC), feature importance is performed for 30 different serum parameters in function of a binary target variable representing either steatotic liver (> 5 % proton density fat fraction) or healthy liver (< 5 %). After hyperparameter tuning, the top 6 relevant features for MASLD are used as the input for a Bayesian Network. Using 5 different structure learning criteria, the 5 best networks are selected, which best describe the relationships between the serum parameters and MASLD.

Results The best RFC (with an ROC AUC of 81 % in the training set) identifies SHBG, ALT, triglycerides, C-reactive protein, HDL cholesterol and GGT as having

the highest impact on MASLD. The five best networks show a range of 7 to 10 (causal) dependencies between the 7 variables, with 2/5 models showing triglycerides, SHBG and HDL cholesterol leading directly towards MASLD. The other biomarkers are either connected to MASLD or a result of MASLD.

Conclusion Bayesian Networks are explainable methods, determining relationships between variables. These may be contrasted with expert knowledge to provide a more complete picture surrounding disease progression. Ultimately, this network will be used to predict MASLD patients in the general population.

P3.03 Discriminating between MASLD and MetALD: Insights from UK Biobank Lipidomics

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DOI 10.1055/s-0043-1777563

Objective Metabolic dysfunction-associated steatotic liver disease (MASLD) and Metabolic dysfunction-associated Alcohol-related Liver Disease (MetALD) represent distinct subsets of liver pathology. Given that the primary distinguishing feature between the two is often based on self-reported alcohol consumption, this study aimed to explore the potential of the lipidomic profile as a differential biomarker.

Methods Utilizing the UK Biobank dataset, 40,534 MRI liver scans were analyzed. 11,217 (27,7 %) cases with a proton density fat fraction (PDFF) ≥ 5 % were identified as having steatotic liver disease (SLD). Among these, lipidomic profiles were obtained for 5,426 MASLD and 644 MetALD cases. 250 lipidomic parameters were measured in the UK Biobank under access number 71300 and analyses were corrected for age,sex and BMI.

Results Among the Top 15 differentially expressed lipidomic markers, all were related to HDL and were significantly overrepresented in MetALD cases. The top 10 metabolites were: Cholesterol in Medium HDL, Cholesteryl Esters in Medium HDL, Total Lipids in Medium HDL, Concentration of Medium HDL Particles, Free Cholesterol in Medium HDL, Apolipoprotein A1, Phospholipids in Medium HDL, Phospholipids in Small HDL, Phospholipids in Large HDL, and Total Lipids in Small HDL.

Conclusion Our findings indicate that HDL-centric lipidomic markers, particularly those within the Medium HDL subfraction, may significantly differentiate MetALD from MASLD. While moderate alcohol consumption is known to raise HDL levels, the underlying mechanisms are multifaceted, involving ethanol metabolism and various biochemical pathways. Further longitudinal and experimental studies are warranted to validate these findings and assess their clinical implications.

P3.04 Progression of MASLD goes along with significant changes of a recruited and TGFb sensitive macrophage population

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The course of fatty liver disease progresses from liver steatosis to chronic inflammation, potentially leading to development of liver cirrhosis, the major risk factor for hepatocellular carcinoma. Cells of innate immunity, especially macrophages, play an important role in this context, although the detailed mechanisms of cell communication are incompletely understood.

The results of the study of a high temporal resolution feeding series prove that the development of fibrosis is accompanied by accumulation of macrophages and formation of so-called crown like structures (CLS) that contain recruited macrophages. A detailed analysis of changes in the composition of immune cell populations during disease progression using CITE Seq Analyzes showed that there is a significant increase in a recruited macrophage population that highly expresses CD11b and CD14. The activation state of this population is substantially controlled by TGFb. The increase in this population is accompanied by a decrease in other macrophage populations in the liver, particularly sessile macrophages. Interestingly, the gene expression of the CD11b-high-CD14-high macrophage population is significantly more influenced by signals via the TGFbRII than the gene expression of the other macrophage populations. This suggests that CD11b-high, CD14-high expressing macrophages play an important role in responses to increased concentrations of active TGFb. These data suggest that the progression of MASLD is accompanied by significant changes in the composition of the liver's macrophage populations, with accumulation of a recruited CD14-high expression macrophage population in particular, whose activation state is relevantly determined by TGFb.

P3.05 Elevated FGF21 serum levels allow the non-invasive detection of NASH with significant fibrosis in NAFLD patients

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Background Non-alcoholic steatohepatitis (NASH) with significant fibrosis (≥ F2) represents a major prognostic factor in non-alcoholic fatty liver disease (NAFLD). Identification of patients at risk for NASH with fibrosis ≥ F2 is therefore important. Although the established fibrosis score FIB-4 is suitable to exclude advanced fibrosis, it does not allow the prediction of significant fibrosis in NAFLD patients. We therefore analysed whether fibroblast growth factor 21 (FGF21), a stress-inducible metabolic regulator, can be used as a non-invasive marker to identify at-risk patients in NAFLD.

Methods We analysed FGF21 levels in sera from an exploration (n = 137) and a validation (n = 88) cohort of biopsy-proven NAFLD patients with different disease activity and fibrosis stages by enzyme-linked immunosorbent assay. In addition, we evaluated whether the use of FGF21 in patients with low (< 1.3) or intermediate (1.3 – 2.67) FIB-4 could identify at-risk patients in NAFLD.

Results FGF21 levels correlated with disease activity and fibrosis in NAFLD and were significantly higher in NASH compared to non-alcoholic fatty liver (NAFL) patients, regardless of the presence of type-2 diabetes. Moreover, FGF21 levels could significantly discriminate between NASH with fibrosis ≥ F2 and NAFLD patients without significant fibrosis, even in patients with low or intermediate FIB-4.

Conclusion Serological FGF21 detection might be useful for the identification of patients at risk for progressed NAFLD.

P3.06 Loss of the mechanistic target of rapamycin complexes 1 (mTORC1) causes a lethal alpha-1 antitrypsin deficiency associated liver disease

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DOI 10.1055/s-0043-1777566

Background and aims PiZ mutation in alpha1-antitrypsin (AAT), a hepatocyte-made, secreted protein, causes AAT deficiency (AATD), a proteotoxic disease characterized by hepatocellular AAT accumulation. Since mTOR is a key regulator of proteostasis, we are investigating the role of mTOR signaling in AATD-related liver disease.

Methods PiZ mice, an experimental AATD model, were crossbred with rodents deficient in hepatocellular mTOR or its interaction partners RAPTOR (mTOR comples 1) or RICTOR (mTOR complex 2) as established proteostatic regulators.

Results 10 weeks old PiZ RaptorΔhep mice, but not PiZ RictorΔhep mice exhibited a lethal liver injury, while PiZ mTORΔhep mice showed a non-lethal phenotype. No increased AAT accumulation was seen. While PiZ RaptorΔhep displayed elevated apoptosis and increased levels of the pro-apoptotic protein CHOP, CHOP ablation did not rescue the phenotype. Serum proteomics revealed no obvious signs of impaired hepatocellular synthesis and PiZ RaptorΔhep mice harbored only minimal fibrosis. Metabolomic analysis revealed profound alterations in arginine metabolism as well as increased glutamate levels. Accordingly, spatial proteomics demonstrated an altered liver zonation that was particularly pronounced in pericentral hepatocytes and likely resulted in lethal hyperammonemia. The pericentral alterations included a decrease in cell-type specific pathways, for instance diminished glutamine synthetase and cytochrome P450 2E1, as well as ectopic activation of periportal processes such as oxidative phosphorylation. This is supported by changes in major regulatory pathways, i.e. Wnt or HNF4α.

Conclusion Our findings demonstrate that Raptor signaling constitutes an essential guardian of metabolic zonation and protects PiZ mice from lethal hyperammonemia.

P3.07 Protease-activated receptor 4: A key player in the progression of metabolic-induced steatohepatitis and tumor development

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The prevalence of metabolically induced fatty liver disease as a risk factor for the development of liver fibrosis and cirrhosis as well as hepatocellular carcinoma (HCC) has increased steadily in recent years. The molecular processes that drive fatty liver disease towards development of inflammation and its progression towards fibrosis, cirrhosis and HCC development are poorly understood.

To examine the relevance of PAR4 in this context mice deficient for PAR4 and wt mice were fed high-calorie or standard diets for up to 50 weeks.

The data suggest that PAR4 is a critical factor for disease progression towards development of fibrosis and HCC. This coincides with significant changes in bile acid composition and the expression of MASLD-associated factors such as GPNMB and IL33 as well as the DAMP molecule HMBG1.

In conclusion these data suggest that PAR4 may be a suitable target for therapeutic intervention to prevent or delay progression of fatty liver disease.

P3.08 The Differential Impact of Animal and Vegetable Fat Intake on the Risk of Metabolic-dysfunction Associated Liver Disease

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DOI 10.1055/s-0043-1777568

Background Approximately every third citizen in Western civilizations is affected by MASLD. While the benefit of a pure vegan diet on MASLD development has already been shown, the relative contributions of animal and vegetable fats remain elusive. We aimed to investigate their comparative impact in relation to total fat intake.

Methods We used a 24h food data from the UK Biobank (n > 210,000) after exclusion of people with pre-existing liver diseases and examined the impact of higher animal- and vegetable fat proportions to the overall fat intake on ICD-10-coded liver diseases. The analyses were adjusted for age, sex, BMI, Townsend-index, kcal, alcohol- and carbohydrate intake.

Results We observed that a higher proportion of vegetable fat is associated with lower odds of ICD10-coded MASLD (OR = 0.643 (0.425-0.973), p = 0.037) as well as liver diseases overall (OR = 0.740 (0.597-0.917), p = 0.006) and liver failure (OR = 0.258 (0.093-0.713), p = 0.009). Conversely, a higher proportion of animal fat intake was associated with increased risk for these conditions ((MASLD: OR = 1.555 (1.027-2.355), liver disease overall: OR = 1.352(1.090-1.676) and liver failure: OR = 3.876(1.402-10.713)). Cox-proportional hazard models showed a lower risk of overall (OR = 0.786, p > 0.001) and digestive-disease related (OR = 0.295, p = 0.001) death for higher vegetable fat intake versus an increased risk when consuming more animal fat (OR = 1.273 and OR = 3.393).

Conclusion Our study provides compelling evidence that a greater proportion of fats from vegetables in our diet may confer protective effects against MASLD and related hepatic conditions. On the contrary, higher consumption of fats from animal sources appears to elevate the risk of MASLD.

P3.09 Accumulation of regulatory T cells correlate with severity of NASH in patients and mice

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DOI 10.1055/s-0043-1777569

Non-alcoholic fatty liver disease (NAFLD) affects approximately 30 % of the world's population. NAFLD is associated with common metabolic comorbidities such as obesity, insulin resistance, type 2 diabetes mellitus, hyperlipidaemia, hypertension and metabolic syndrome and 10-20 % of NAFLD patients develop non-alcoholic steatohepatitis (NASH), which can progress to hepatocellular carcinoma (HCC). Therefore, although the majority of NAFLD patients die from cardiovascular events rather than liver-related causes, NAFLD is an increasing cause of fatal disease worldwide.

Animal models should therefore mimic these disease conditions to advance our understanding of pathophysiology and new therapeutic interventions. A high-fat/high-carbohydrate diet, including fructose, is currently one of the best ways to induce a NASH phenotype in otherwise healthy inbred mouse strains. Furthermore, to prove the relevance of our experimental data we additionally retrospectively quantified the T cell compartment in liver biopsies and peripheral blood samples from NASH patients.

We analyzed the intrahepatic Treg response and the impact of therapeutic interventions that increase intrahepatic Treg numbers in experimental NASH with a strong activation and clonal expansion of intrahepatic T cells . Lobular accumulation of regulatory T cells increases with severity of NAFLD in humans. In association with increasing metabolic inflammation, Tregs differentiate into TH17-like cells, promoting disease exacerbation.

In conclusion, T cells are the drivers of metabolic inflammation. Nonetheless, Tregs are not able to control this inflammation but promote disease exacerbation by converting to TH17-like cells.

P3.10 BMP-9 acts as a homeostasis factor balancing the gut-liver cross-talk in mice and men

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Bone morphogenetic protein (BMP)-9, a member of the TGFβ-family of cytokines, is mainly produced in the liver and constitutively circulates in the blood of healthy individuals. In healthy liver, BMP-9 stabilizes the differentiated phenotype of hepatocytes and the endothelium. The serum levels of BMP-9 are reduced in diabetic patients, and BMP-9 overexpression ameliorates steatosis in the high-fat-diet-induced obesity mouse model.

During the process of liver regeneration in mice, BMP-9, as an antagonist cellular plasticity, is transiently down-regulated. We now confirmed that such transient down-regulation (serum level) also occurs in patients directly after large liver operations (n = 28 patients). Recently we showed that besides the liver, the small intestine is also a source for BMP-9 and specifically intestinal BMP-9 expression negatively correlates with diabetes in adipose patients. Using intestinal organoids, we now add that BMP-9 strongly supports expression of differentiation markers in the intestine, whereas stemness markers like LGR5 are down-regulated. Finally, we started breeding a macrophage-specific Alk1 KO mouse line. Alk1 is the BMP-9 receptor with highest affinity to the ligand, which we found highly expressed on macrophages. Interestingly, these KO mice are significantly heavier than their wild-type siblings, implying that at least some of BMP-9s' actions on metabolism are mediated via Alk1 signalling in macrophages.

In summary, our data demonstrate that BMP-9 controls the physiological differentiated state of liver and intestine, and thereby acts protective against development of metabolic derailments that precede pathologic states like diabetes and NASH.

P3.11 Carbohydrate response element binding protein (ChREBP) correlation and contribution to NAFLD-mediated hepatocarcinogenesis

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Backgroud Carbohydrate-response-element-binding-protein (ChREBP) is a glucose-mediated transcription-factor that is mainly expressed in liver & strongly involved in glycolytic & lipogenic-pathways [1, 2]. ChREBP has emerged as a pioneer-factor for regulation of de-novo lipogenesis (DNL), an important source of fatty-acids in the development of NAFLD (non-alcoholic-fatty-liver-disease) [3]. Previous studies from our group have suggested an associative role of ChREBP in development of hepatocellular-carcinoma (HCC) [4,5].

However, the molecular-pathogenesis of ChREBP-related-hepatocarcinogesis in response to high-fat-diet (HFD) in mice model remains unexplored. Here, we want to explore, role of PI3K/AKT/mTOR & RAS/RAF/MAPK signaling-pathways in liver-pathogenesis of our experimental mice models.

Methods Male C57BL/6J (WT), liver-ChREBP-KO (L-KO) & total body-ChREBP-KO (TB-KO) mice were maintained on either a HFD-(46 %-fat) or control-diet (10 %-fat) for 3 ,6 & 12-months to induce metabolic syndrome. Parallel mice-groups, with single-injection (at 4-weeks-old) of potent-hepatocarcinogen DEN (Diethylnitrosamine), were used as HCC-reference groups. Body-weight & blood-glucose were measured once a month. Post-perfusion, frozen liver-tissue samples were examined by western-blotting & RT-PCR.

Results We have investigated the role of PI3K/AKT/mTOR & RAS/RAF/MAPK pathways & their correlation with ChREBP-hepatic-deficiency. Our molecular-data suggest a notable downregulation in PI3K/AKT/mTOR (mTOR, AKT2, MAPK1) & RAS/RAF/MAPK pathway (HRAS, IRS1) as well as glycolysis (MLX, PKM) & lipogenesis (FASN) after ChREBP-knockout, compared to WT-mice, in both mice-models (HFD & DEN).

Conclusion Our present data further support the suggestion of a proto-oncogenic function of the transcription-factor ChREBP. Furthermore, our on-going study suggest the absence of HCC-development after liver-ChREBP deletion as well as downregulation of glycolytic & lipogenic-profile of the liver.

P3.12 Deciphering the role of ferroptosis in non alcoholic fatty liver disease

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DOI 10.1055/s-0043-1777572

Non alcoholic fatty liver disease (NAFLD) represents a leading cause of chronic liver damage, which can progress to non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC). It is already known that in the pathogenesis of NAFLD, increased hepatic lipid accumulation results in augmented oxidative stress, which increases organ damage. Therefore, inhibition of ferroptosis,a programmed cell death mechanism based on triggering oxidative stress, is a potential therapeutic option for patients with NAFLD.

In this work, we intended to determine whether a liver parenchymal cell (LPC)-specific conditional deletion of acyl-CoA synthetase long-chain family member 4 (Acsl4LPC-KO) can influence the onset and progression of NAFLD. For this purpose, wild-type (wt) and Acsl4LPC-KO animals were fed a choline deficient high fat diet (CD-HFD) for 20 and 40 weeks, illustrating different stages of metabolic liver damage from NAFLD to cirrhosis with HCC progression as well as the development of metabolic syndrome. Subsequently, the mice were subjected to metabolic analyses.

In contrast to the recently published data by Duan et al. (PMID:34510514), we found no significant differences between Acsl4LPC-KO and wt littermates, neither in the development of NAFLD nor in progression of metabolic syndrome. Moreover, there were no differences in metabolic analyses (weight gain, glucose tolerance test, hepatic steatosis) or NAFLD-associated inflammatory response.

According to our analyses, deletion of Acsl4 and concomitant inhibition of ferroptosis appears to have no effect on NAFLD. Through further lipidome analyses, we aim to identify possible differences in the fat metabolism between Acsl4LPC-KO and wt mice that could explain the diverse phenotype.

P3.13 Fatty liver diseases are more common in post-COVID-syndrome

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Background After acute infection with SARS-CoV-2, a significant proportion of patients suffer from post-COVID. Pathomechanisms underlying the development of these sequalae are still poorly understood, several studies suggest an increased risk in patients with comorbidities. Chronic liver disease may play a relevant role, as frequent symptoms like fatigue can occur in both, post-COVID and chronic liver disease. Therefore, the aim of our study was to evaluate the frequency of steatosis hepatis in patients suffering from post-COVID syndrome

Methods Patients presenting in the post-COVID outpatient department of Jena University Hospital between July 2020 and August 2022 were evaluated with ultrasound and transient elastograpy (FibroScan), neuropsychological tests (fatigue screening, MOCA) and laboratory analyses (including IL-6, CRP, ferritin).

Results A total of 599 patients were included in the analysis, 373 (62.3 %) presented findings of steatosis hepatis in transient elastography. Steatosis hepatis was more frequent in male patients (41.6 vs. 24.8 % $p < 0.001$) and was associated with age (54 vs. 45 years, $p < 0.001$) and higher BMI (29.3 vs. 23.7 kg/m2, $p < 0.001$) as expected. There were no differences in neurocognitive screening in relation to fatigue, but more cognitive dysfunction was identified via MoCA in patients with steatosis compared to non-steatosis (36.0 vs. 23.2 %, $p = 0.003$).

Conclusion Fatty liver diseases are more frequent in post-COVID patients than in the general population and associated with fatigue and cognitive dysfunction in this population. However, further studies are needed to investigate long-term sequelae.

P3.14 Reciprocal Inhibitory Regulation of TGF-β1 Signaling and Cholesterol Metabolism in Hepatocytes

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Objective Transforming growth factor-β1 (TGF-β1) plays important roles in metabolic dysfunction-associated steatotic liver disease (MASLD), which involves various biological processes including dysfunctional cholesterol metabolism contributing to progression to metabolic dysfunction-associated steatohepatitis (MASH) and hepatocellular carcinoma (HCC). However, how TGF-β1 signaling and cholesterol metabolism affects each other in MASLD is yet unknown.

Design Changes in gene transcriptions associated with cholesterol metabolism were assessed by RNA-Seq of AML12 cells and mouse primary hepatocytes (MPH) treated with TGF-β1. Functional assays were performed on AML12 cells (untreated, TGF-β1 treated, or subjected to cholesterol enrichment (CE) or depletion (CD)), and on mice injected with AAV8-Control/TGF-β1.

Results TGF-β1 inhibited mRNA expression of several cholesterol metabolism regulatory genes, including rate-limiting enzymes of cholesterol biosynthesis in AML12 cells, MPHs, and AAV8-TGF-β1-treated mice. Total cholesterol levels in AML12 cells, as well as lipid droplet accumulation in AML12 cells and AAV-treated mice were also reduced. Smad2/3 phosphorylation following 2 h TGF-β1 treatment persisted after CE or CD and was mildly increased following CD, while TGF-β1-mediated AKT phosphorylation (30 min) was inhibited by CE. Furthermore, CE protected AML12 cells from several effects mediated by 72 h incubation with TGF-β1, including EMT, actin polymerization, and apoptosis. CD mimicked the outcome of long term TGF-β1 administration, an effect that was blocked by an inhibitor of the type I TGF-β receptor. Additionally, the supernatant of CE- or CD-treated AML12 cells inhibited or promoted, respectively, the activation of LX-2 hepatic stellate cells.

Conclusion TGF-β1 inhibits cholesterol metabolism while cholesterol attenuates TGF-β1 downstream effects in hepatocytes.

P3.15 Synergistic influence of asthma and obesity on hepatic metabolism in a mouse model

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Background Obesity is a widespread disease with increasing case numbers and serious health consequences. Asthma is the most common chronic respiratory disease. Asthma and obesity frequently co-occur and usually negatively influence the respective disease course. Recent epidemiological studies suggest a pathomechanistic link between obesity and asthma. Molecular biology studies on the mechanism of this interaction, however, are missing.

Methods Four groups of C57BL/6 mice (n = 6 animals each) were fed either a high-fat diet (HFD) or normal diet (ND) until 20 weeks of age. During the last 4 weeks mice were additionally treated by repeated intranasal applications of house dust mite extract (HDM) or PBS as a control. Liver samples were analyzed by qRT-PCR and immunohistochemistry for biochemical markers of glucose metabolism and inflammatory parameters.

Results In all groups with HFD or HDM exposure, genes of glucose metabolism were regulated. In particular, expression of glycolysis associated enzymes such as phosphofructokinase 1 and aldolase were decreased in the HDM/HFD group. In addition, a decrease of succinyl-CoA synthetase was detected in liver tissue by both, qRT-PCR and immunohistochemistry. While IL-10 as a parameter of hepatic anti-inflammatory immune response was increased in the HDM group, markers for Th1 immune response were downregulated in the HDM/HFD group.

Conclusion Our results demonstrate the potentiated morbidity of asthma and obesity on parameters of hepatic glucose metabolism and hepatic inflammation. Therefore, asthma and obesity show a synergistic negative impact on liver function and thus increasing liver injury.

P3.16 Patients with Liver cirrhosis suffer from functional cobalamin deficiency despite sufficient cobalamin serum levels

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Introduction Liver cirrhosis is a complex disease associated with an increased risk of malnutrition, particularly vitamins and minerals. Interestingly in contrast to these findings, cirrhosis patients often exhibit normal or even elevated serum levels of vitamin B12. However, the extent to which a functional cobalamin deficiency exists despite adequate cobalamin plasma levels in these individuals has not yet been sufficiently investigated. Therefore, the aim of the present study was to determine functional cobalamin markers in serum of cirrhosis patients.

Methods Fifty-four patients with liver cirrhosis (Child-Pugh-Score: A:(n = 1), B:(n = 26), C:(n = 27); male:(n = 43), female:(n = 11), etiology: alcohol:(n = 49), others:(n = 5) and a control group of 31 subjects without cirrhosis (male:(n = 11), female:(n = 22)) were included. We determined the most important cobalamin associated metabolic markers in all participants such as serum levels of vitamin B12, holo-transcobalamin, methylmalonic acid (MMA), folic acid, vitamin B6 and homocysteine.

Results Compared to controls (Ctrl), patients with cirrhosis (LC) exhibited significantly higher levels of vitamin B12 (642pmol/l vs. 298pmol/l) and holo-transcobalamin (159pmol/l vs. 98pmol/l). Interestingly, the serum MMA levels in LC were significantly higher than in Ctrl (256nmol/l vs. 183nmol/l). Conversely, homocysteine levels (18µmol/l vs. 22µmol/l) were lower in LC compared to Ctrl ($p < 0.05$). All other markers did not differ between groups.

Conclusion The results suggest the presence of a functional cobalamin deficiency in liver cirrhosis patients despite normal B12 serum levels. On the other hand, the sole determination of B12 serum levels appears to be inadequate. Therefore, future clinical practice should include the assessment of additional functional markers such as MMA.

P3.17 Deep learning-based predictive modeling of PNPLA3 variant carriers using radiomics data from the UK Biobank

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Background Steatotic liver disease (SLD) represents a prevalent public health concern.Genetic factors play a pivotal role in SLD's development, especially the patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene, with its most prominent variant rs738409_G. Identification of rs738409_G carriers based on genome analysis is expensive. Therefore, we propose a novel approach to discern rs738409_G carriers utilizing liver magnetic resonance imaging (MRI) data.

Methods We selected UK Biobank patients for which both data on PNPLA3 rs738409_G status (N_homozygous = 2041, N_non-carrier = 26756) and MRI data was available. To extract the liver fat fraction we calculated r2*-corrected

water and fat images based on the IDEAL post-processing technique . The liver was subsequently segmented by training a UNet model and radiomics features were extracted. We then trained a Random Forest classifier using extracted radiomics features for a matched group of non-carriers and homozygotes to predict homozygous carriers and non-carriers.

Results The data was divided into the fatty liver group (1018 controls and carriers, respectively) and normal liver fat group (803 controls and carriers each) based on the fat content (threshold 5 %) and the prediction achieved an AUROC of 0.62 and 0.54 on the independent test sets respectively, which indicates that the livers of SLD patients contain more features to differentiate between homozygous carriers and non-carriers.

Conclusion Utilizing a well-characterized and large cohort, we demonstrate a novel approach to identify PNPLA3 rs738409_G carriers. Our approach introduces a streamlined and accessible method for identifying carriers, which holds great promise for advancing the field of SLD diagnostics and personalized medicine.

P3.18 Characterizing Molecular Pathways of Precancerous Changes in Hepatocytes Under Metabolic Overload

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Background Hepatocellular carcinoma (HCC) is a leading cause of cancer-related deaths, often diagnosed late with a poor prognosis. This study aimed to elucidate molecular processes associated with precancerous alterations arising from metabolic overload in hepatocyte like cells, shedding light on potential pathomechanisms underlying non-cirrhotic NAFLD progression and hepatocarcinogenesis.

Methods Differentiated HepaRG cells, as modell of unaltered hepatocytes, were subjected to in vitro treatments mimicking metabolic syndrome by exposing them to excess insulin, glucose, and free fatty acids (FFA) up to 48h. mRNA expression of key genes involved in fat and glucose metabolism, and the tumor related genes PTEN, TP53, Abl1, ANXA2-4, and SIRT1were quantified via real-time PCR. Intracellular protein expression and phosphorylation were analyzed through Western blotting for genes related to cell cycle control (PCNA, E2F) and PI3K signaling (NF-kappaB, PTEN).

Results Robust cellular steatosis of fully differentiated HepaRG cells was detected under treatment with FFA and combined glucose, insulin, and FFA treatment. However, no significant changes in mRNA expression of the analyzed genes was detected.

Conclusion Short term steatosis seems not to affect expression or regulation of genes involved in fat or glucose metabolism, tumor suppression, or growth regulation. Long-term effects of metabolic overload should be analyzed in appropriate models to estimate its impact on non-cirrhotic tumorigenesis.

P3.19 Metabolische Veränderungen und deren Zusammenhang mit dem Leberphänotyp bei Erwachsenen mit homozygotem Alpha1-Antitrypsin-Mangel (Pi*ZZ-Genotyp)

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Einleitung und Ziel Der schwerwiegende Alpha1-Antitrypsin-Mangel (Pi*ZZ) prädisponiert zur Lungen- und Lebererkrankung. Da das Ausmaß der Leberbeteiligung erheblich variiert, haben wir die Zusammenhänge zwischen metabolischen Faktoren und dem Leberphänotyp in Pi*ZZ-Individuen untersucht.

Methodik Es wurde eine multizentrische, internationale Kohorte von 707 Pi*ZZ-Individuen und 329 Kontrollen (Pi*MM) untersucht. Alle Studienteilnehmer unterzogen sich einer umfassenden Leberuntersuchung sowie der Messung der Lebersteifigkeit (LSM) mittels transienter Elastographie (Fibroscan).

Ergebnis Pi*ZZ-Individuen wiesen im Vergleich zu Pi*MM-Teilnehmern erhöhte Serumwerte für ALT, AST, GGT, GLDH und AP auf. Die Prävalenz einer signifikanten (LSM ≥ 7,1 kPa) bzw. fortgeschrittenen Leberfibrose (LSM ≥ 10 kPa) war in Pi*ZZ-Trägern 4,4-fach (24,4 % vs. 5,6 %) bzw. 8,1-fach (10,5 % vs. 1,3 %) höher im Vergleich zu Kontrollen. Obwohl die Anzahl der Diabetiker in beiden Gruppen vergleichbar war und Pi*ZZ-Träger einen niedrigeren Body-Mass-Index (BMI) (25,3 kg/m2 vs. 26,1 kg/m2, p = 0,011) aufwiesen, zeigten Pi*ZZ-Teilnehmer signifikant häufiger HOMA-Werte ≥ 2,5 (41,3 % vs. 27,4 %, p = 0,003). Im Gegensatz dazu waren ihre Triglycerid- (99,2 vs. 130,6 mg/dl, p < 0,001) und Cholesterin-Spiegel (208,4 mg/dl vs. 215,4, p = 0,002) niedriger. Pi*ZZ-Träger mit signifikanter Leberfibrose präsentierten erhöhte Triglycerid-, HbA1c-, HOMA- und C-Peptid-Werte sowie häufiger ein metabolisches Syndrom und metabolisch assoziierte Fettlebererkrankung/Stoffwechselstörung-assoziierte steatotische Lebererkrankung (MAFLD/MASLD) (Tabelle 1). Darüber hinaus traten männliches Geschlecht (OR = 4,25), HOMA-Werte ≥ 2,5 (aOR = 2,98), Triglyceride ≥ 150 mg/dL (aOR = 2,03), BMI ≥ 30 kg/m2 (aOR = 3,18) und Diabetes (aOR = 2,14) bei Pi*ZZ-Trägern mit LSM ≥ 7,1 kPa überproportional häufig auf.

Schlussfolgerung In Pi*ZZ-Individuen bestehen signifikante Assoziationen zwischen metabolischen Faktoren und dem Vorliegen einer signifikanten Leberfibrose. Die erhöhten HOMA-Werte deuten auf eine Insulinresistenz hin.

P3.20 ABHD5 overexpression protects PNPLA3-148M primary human hepatocytes from steatosis in liver chimeric mice with NAFLD

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Background and Aims The methionine variant of patatin-like phospholipase domain-containing protein 3 (PNPLA3-148M) is associated with advanced fatty liver disease. Previous studies have proposed a mechanism where PNPLA3-148M sequesters alpha/beta-hydrolase domain containing 5 (ABHD5), thereby impairing adipose triglyceride lipase (ATGL) activity on lipid droplets. Here we test the hypothesis that ABHD5 overexpression in primary human hepatocytes (PHH) with PNPLA3-148M can overcome sequestration and improve steatosis in liver chimeric mice with NAFLD.

Methods PHH from a homozygous PNPLA3-148M donor (PHH-148M) were lentivirally transduced with ABHD5 prior to transplantation into immunodeficient Fah-/- liver injury mice. To link the PNPLA3 genotype to advanced steatotic liver disease, PNPLA3-148M and ABHD5 were co-transduced in PHH from a homozygous PNPLA3-148I donor (PHH-148I). After reaching peak liver humanization animals were challenged with a Western-style diet (WD, 60 % kcal from lard, 23 % sucrose, 0.03 % cholesterol) for four weeks.

Results PHH-148M developed more severe steatosis in response to WD than PHH-148I, which was confirmed in PNPLA3-148M transduced PHH-148I that also displayed widespread microvesicular steatosis. ABHD5 overexpression in PHH-148I did not affect steatosis. In contrast, ABHD5 overexpression in PHH-148M resulted in > 4-fold lower steatosis compared to control mice. While ABHD5 minimally improved steatosis in PNPLA3-148M transduced PHH-148I, it nearly completely resolved microvesicular steatosis.

Conclusion These findings support a model where sequestration of ABHD5 by PNPLA3-148M impairs triglyceride lipolysis by ATGL, leading to excess steatosis upon hypercaloric feeding. Overexpression liver chimeras with PHH-148I or

PHH-148M grafts provide mechanistic insights on how PNPLA3 and ATGL maintain human lipid homeostasis under hypercaloric conditions.

P3.21 Serum proteomic signature of alcohol detoxification and the role of PNPLA3 genotype

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Introduction Alcoholic liver disease (ALD) is the leading cause of liver related death, but its mechanisms remain incompletely understood. To change that, we studied proteomic changes in subjects undergoing inpatient alcohol detoxification with a focus on the role of PNPLA3 variant as the major genetic ALD modifier.

Methods Sera of 95 ALD subjects (44 PNPLA3WT/WT, 20 PNPLA3I148M/I148M) were collected prior to and after undergoing inpatient detoxification (median duration: 6 days) and were compared to sera of 32 liver-healthy controls. All samples were subjected to untargeted mass spectrometry-based proteomic analysis. Based on liver stiffness measurements via transient elastography (cut-off 7.5 kPa), the ALD subjects were subdivided into a lower (n = 57) and advanced (n = 45) fibrosis group.

Results The proteomic pattern of ALD subjects strongly differed from healthy controls and changes were more pronounced in subjects with advanced vs. low fibrosis. Complement factor 7 (C7), galectin 3-binding protein (LGALS3BP) and polymeric immunoglobulin receptor (PIGR) were among the most prominent species elevated in advanced disease stages while inter-alpha-Trypsin Inhibitor Heavy Chain 1 (ITIH1) and complement factor 6 (C6) were diminished. Detoxification resulted in a partial recovery of these features (e.g. C6, C7, PIGR), while some proteins displayed an even stronger abnormality (von Willebrand factor). Compared to PNPLA3WT/WT, PNPLA3I148M/I148M subjects harbored more pronounced changes in apolipoproteins suggestive of stronger lipidomic alterations.

Conclusion Our data provide novel insights into ALD-related alterations and the role of the PNPLA3 variant in this process.

Keywords Alcohol-Related Liver Disease (ALD), Proteomic Profiling, PNPLA3 Genotype, Apolipoproteins, APRI, fibrosis

P3.22 Relationship of non-invasive measures with histological response in patients with nonalcoholic steatohepatitis and fibrosis: 52-week data from the Phase 3 MAESTRO-NASH trial

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Background MAESTRO-NASH (NCT03900429) is an ongoing 54-month, randomized, double-blind, placebo-controlled Phase 3 trial evaluating the efficacy of resmetirom in patients with biopsy-confirmed nonalcoholic steatohepatitis (NASH) and fibrosis. 966 patients with biopsy-confirmed NASH were randomized 1:1:1 to resmetirom 80mg, resmetirom 100mg, or placebo administered once daily. Histologic endpoints were assessed after 52 weeks. Dual primary endpoints at Week 52 were achieved with both resmetirom 80mg and 100mg: NASH resolution with no worsening of fibrosis (NR) or ≥ 1-stage reduction in fibrosis with no worsening of NAS (FR).

Methods The relationship of non-invasive measures with histological response (NR and/or FR) in the resmetirom 80mg, resmetirom 100mg, and placebo groups was assessed.

Results Patients with biopsy-confirmed NASH with fibrosis had high metabolic risk. Among patients treated with resmetirom 80mg or 100mg who achieved a ≥ 30 % reduction from baseline in MRI-PDFF, NR was observed in 28 % and 38 % and FR in 17 % and 18 % more patients than placebo. A ≥ 30 % PDFF response was observed in 96 %, 88 %, and 92 % of resmetirom 100mg NR, FR, and NR and/or FR responders. Half of resmetirom ≥ 30 % PDFF responders without NR or FR showed ≥ 2-point NAS reduction. On biopsy, NR correlated (r2) with FR (= 0.30). Additional correlates (r2) of NR and FR at resmetirom 100mg included reduction in PDFF (0.39, 0.23); ALT (0.20, 0.24); and liver volume (0.25, 0.18).

Conclusion Achievement of NASH resolution and fibrosis reduction was associated with a ≥ 30 % reduction from baseline in MRI-PDFF – at both resmetirom doses (80 and 100mg). Additional analyses are ongoing.

P3.23 Loss of the p38 MAPK-activated protein kinase MK2 does not significantly alter the progression of metabolic dysfunction-associated steatotic liver disease (MASLD) in mice on high-fat/high-sugar diet (HFSD)

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DOI 10.1055/s-0043-1777583

Background MK2 is a direct target of the stress-activated MAPK p38 and regulates inflammatory processes. MK2-deficient mice are protected in inflammatory disease models, which confirmed the kinase as interesting therapeutic target. Since MASLD is associated with low grade chronic inflammation, we analyzed the progression of the disease in MK2-deficient mice.

Methods Wildtype and MK2-deficient mice were fed a HFSD for 16 or 24 weeks. Body, liver, epididymal fat weight and non-fasted blood glucose levels were documented. TD-NMR analysis determined lean/fat mass as well as free body fluid. Liver steatosis, inflammation, potential fibrosis, and alterations of the adipose tissue were analyzed by immunohistochemistry. Expression of metabolic and inflammatory markers were monitored by qPCR analyses of liver and fat tissue.

Results MK2-deficient mice were significantly lighter than wildtype mice after 16 and 24 weeks of HFSD, mainly due to less lean mass and free body fluid rather than fat mass. Accordingly, liver weight, epididymal fat mass and blood glucose levels were not significantly altered. No significant difference in adipocyte size, crown-like structures or the expression of inflammation markers was detectable in adipose tissue. Similarly, quantification of the Sudan IV-positive lipid rich area in the liver and qPCR analysis of metabolic and inflammatory markers in liver tissue did not display significant alterations between wildtype and MK2-deficient mice.

Conclusion Despite its known function in the regulation of inflammation, the loss of MK2 expression does not significantly alter the progression of MASLD in mice after 16 or 24 weeks of HFSD.

P3.24 Unsaturated fatty acids aggravate mitochondrial dysfunction in senescent primary hepatocytes

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DOI 10.1055/s-0043-1777584

Background and Aim Senescence is an irreversible cell cycle arrest caused by cellular stress leading to a senescent phenotype. In pre-clinical models, the steatotic liver shows increased hepatocellular senescence. Oleic acid (OA), a

monounsaturated fatty acid (FA), prevents saturated FA-induced lipotoxicity but is implicated in MAFLD development. We hypothesize that senescence hinders oleic acid's beneficial effects via mitochondrial dysfunction.

Methods Primary hepatocytes, isolated from mice, were cultured overnight. Cells were sensitized with senescence inducers H2O2 (oxidative stress) and Nutlin 3a (p53 stabilization) for 24 hours, followed by 24-hour incubation with OA. Steatotic or senescent cells were further treated with senolytics, dasatinib, and quercetin (D + Q) for 24 hours. Neutral lipid was measured by Oil Red-O staining and senescence markers p21, p53, and γH2A.X by immunofluorescence. Mitochondrial function was assessed by XFe Seahorse analyser.

Results 24 hours OA-treatment increased lipid accumulation by 4-fold ($p < 0.05$) and upregulated p53, γH2A.X, and p21 (1.25-fold, $p < 0.05$). While OA enhanced metabolic activity in non-senescent cells, it impaired mitochondrial function in senescent cells (H2O2 or Nutlin 3a-treated). Dasatinib and quercetin treatments (senolytics) were able to recover these effects while p53 could be reduced (p-ns) and fat accumulation remained unchanged. However, pre-senescent D + Q treated hepatocytes have shown reduced fat content along with decreased p53 and γH2A.X.

Conclusion Oleic acid induces senescence in primary hepatocytes and enhances OCR and glycolysis, while senescent cells exhibit the opposite response. This highlights the significance of targeting senescence therapeutically in MAFLD.

Poster Visit Session IV TUMORS 27/01/2024, 08.30am–09.10am

P4.01 KAIGI HCC – transsektorale Austauschplattform zur Therapie des HCC

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Leberkrebs ist weltweit die fünfthäufigste Krebsart und die dritthäufigste Krebstodesursache. Die Zahl der Neuerkrankungen könnte bis 2040 um > 55 % steigen. Patienten mit HCC werden anhand spezifischer Variablen in fünf Stadien eingeteilt (BCLC-Score). Für Deutschland sind die Diagnose- und Behandlungskonzepte in drei sich ergänzenden klinischen Leitlinien (HCC 2022, NAFLD 2022, Leber-Transplantation 2023) zusammengefasst. Die Diagnostik und Therapie des HCC ist jedoch komplex. Neben der gleichzeitigen Behandlung von mindestens drei Erkrankungen (Lebergrunderkrankung, Leberzirrhose/Z.n. LTx und HCC) stellen die aktuellen Therapiealgorithmen/Innovationen große klinische Herausforderungen dar. Daraus resultiert die Notwendigkeit einer Behandlung im interdisziplinären/transsektoralen Team. Für die praktikable Umsetzung im klinischen Alltag ergab sich daraus der Wunsch nach einer einfach zu bedienenden Austauschplattform. KAIGI HCC ist eine multidisziplinäre, regionale Austauschplattform zum HCC, die in Zusammenarbeit von Roche und HCC-BehandlerInnen entwickelt wurde . Die Plattform ist login-gesichert und bietet die Möglichkeit, mit SpezialistInnen über Themen rund um das HCC zu diskutieren. Schwerpunkt ist hierbei die leitliniengerechten Versorgung von PatientInnen und umfasst das gesamte Spektrum von Prävention, Screening, Diagnose, Behandlung und Nachsorge im Verbund. Der Austausch auf KAIGI HCC wird durch ein Diskussionsforum, Übersichtsmaterialien und das Teilen klinischer Fälle ermöglicht. Zudem können DSGVO-konforme Videogespräche mit HCC-/Transplantations-ExpertInnen durchgeführt und Termine geplant werden. So wird eine schnellere und unkompliziertere Abstimmung zwischen ZuweiserInnen und ExpertInnen möglich. In einer User-Befragung ergab sich bereits eine durchweg positive Rückmeldung. KAIGI HCC bietet auch für das aktuelle Therapie Migration Konzept eine leitlinienbasierte Unterstützung bei der HCC-Therapieentscheidung. KAIGI HCC kann individuell an die regionalen HCC-Therapiestrukturen angepasst werden und unterstützt das Konzept der transsektoralen

P4.02 Imatinib induces clinical response in a patient with refractory combined hepatocellular and cholangiocellular carcinoma harbouring a rare PDGFRA exon 18 mutation (p.I843_S847delinsT)

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DOI 10.1055/s-0043-1777586

Introduction Combined hepatocellular cholangiocarcinoma (cHCC-CCA) is a rare primary liver malignancy. Currently, there are no guidelines for the management of patients with this disease.

Methods Case report of a patient with a rare PDGFRA mutation receiving fourth-line tyrosine kinase therapy.

Results In 2020, a 50-year-old male patient presented to our department with weight loss and pain in the right upper quadrant. CT and MRI images of the liver showed a diffusely growing tumor involving all liver segments and multiple satellite lesions. Histologically, the patient was diagnosed with cHCC-CCA. Transarterial radioembolization was performed for tumor debulking prior to initiation of systemic gemcitabine/cisplatin therapy. After 15 months, progression was observed for the first time, as well as an increase in alpha-fetoprotein (AFP). Therefore, treatment was switched to a dual checkpoint inhibitor therapy with atezolizumab/bevacizumab. Unfortunately, six months later, the patient again showed progression as well as an increase in the tumor marker CA19-9, and systemic therapy was switched to FOLFIRI. Two months later, the patient presented with lumboischialgia and leg paresis. With radiological evidence of cervical and thoracic metastases, cervical laminectomy, spinal decompression, and tumor resection were performed, followed by local radiation. Genomic sequencing of the tumor revealed a rare mutation in exon 18 of PDGFRA (p.I843_S847delinsT), so we started off-label therapy with imatinib, which has resulted in disease control to date.

Conclusions This is the first case of cHCC-CCA with PDGFRA exon 18 mutation with clinical response to imatinib and highlights the importance of a precise oncologic approach.

P4.03 Inducibility of ferroptosis in cholangiocellular carcinoma cells and the occurrence of para- and autocrine mechanisms

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Cholangiocellular carcinoma (CCA) refers to a malignant degeneration of cholangiocytes and is considered the second most common carcinoma of the liver. Due to the increasing incidence and high mortality rate, there is a perceived urge for new therapeutic approaches. One promising approach is the pharmacological induction of cell death such as the iron-dependent cell death ferroptosis. The aim of this study was to determine the inducibility of ferroptosis in five different CCA cell lines and to investigate the impact of para- and autocrine mechanisms of ferroptotic cells in vitro.

The cell lines served as a model for the heterogeneity of CCA and showed an inhomogeneous response to ferroptosis inducers explained by the pattern of gene expression of various ferroptosis-associated genes, which could be applied as a scoring system also in human tumors. With respect to the secretome emitted by ferroptotic cells, we also found that the secretome of dying cells was capable of inducing cell death itself in the corresponding cell line.

Regarding para- and autocrine mechanisms of ferroptotic cells, we searched for modulatory cell death mediators at both the protein and gene expression levels and, in line with preliminary work of other groups, identified interleukin

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6 as an interesting therapeutic target that can modulate the ferroptosis sensitivity of cells.

In summary, this work indicates that the secretome of ferroptotic cells can have a crucial influence in terms of para- and autocrine mechanisms on neighboring cells, which may provide targets for pharmacological modification of the ferroptotic response of cancer cells.

P4.04 Rare but not forgotten – deep molecular and histological characterization of the liver yolk sac tumor and corresponding primary-derived cell line

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Primary yolk sac tumor (YST) of the liver is uncommon disease with less than 15 cases reported worldwide and is often misdiagnosed as HCC. Therefore, in-depth molecular and histological characterization of YST and development of representative models are essential for better diagnosis and development of patient-specific therapies.

Samples of adjacent liver and tumor tissue were collected and processed after surgery. Long-term culture of the primary cell line (PCL) was established. Morphological and histological characteristics of tissues, xenograft tumors and PCL were analyzed by immunohistochemistry (IHC) and immunofluorescence (IF). Immune cell composition was inferred from RNA-seq data and validated by IHC. Transcriptomic profiling was performed by RNA-seq followed by time-course, GSEA and IPA analyses. Key oncogenic alterations and actionable mutations were identified by NGS and specific inhibitors were applied.

Tumor tissue showed solid trabecular growth pattern. IF and IHC displayed expression of typical YST markers AFP, CK19, which were effectively preserved in xenografts, PCL and spheroids. Transcriptomic profiling revealed activation of embryonic markers, enrichment of T cells and reduction of macrophages. Previously unknown genomic alterations (22q12.2, 9q34.11, 8q24.3 amplifications) were revealed. NGS showed presence of TP53 and KDR mutations which were highly conserved in PCL. Specific targeting of KDR confirmed sustained sensitivity to specific inhibition compared to non-mutated control cells. For the first time, we were able to dissect liver YST at unprecedented molecular level and to establish primary cell line. We identified molecular alterations that could be used for targeted therapy and established cellular model for this extremely rare disease.

P4.05 Treatment reality and outcomes of patients with intermediate, locally advanced or metastatic hepatocellular cancer – Results from the prospective national intersectoral cohort study JADE

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Introduction Prospective real-world evidence on hepatocellular carcinoma (HCC) especially long-term observations are scarce. This concerns clinically relevant questions, particularly regarding the implementation of newly approved systemic treatments into routine clinical care and their impact on patient outcome. Clinical trials often exclude patients with an ECOG performance status (PS) of ≥ 2 or impaired liver function (Child-Pugh score B or C).

Patients and Methods Here, we present results from the prospective, multicenter, intersectoral, longitudinal cohort study JADE (NCT04510740) collecting data on patients with HCC from approximately 100 sites since August 2020 in Germany. Patients and tumor characteristics, systemic treatment patterns and outcome data are presented for patients with intermediate or advanced HCC (aHCC).

Results At database cut (30.04.2023), 197 patients with aHCC received systemic treatment; median age at treatment start was 72 years, 87 % were male, 18 % had an ECOG PS of ≥ 2, 95 % had comorbidities, 47 % had a Child-Pugh score A.

The three most frequent first-line treatments (Aug 2020-Apr 2023) were ATZ + BEV (156/197, 79 %), lenvatinib (20/197, 10 %) and sorafenib (8/197, 4 %). A quarter of patients had already received subsequent second-line treatment, predominately with sorafenib (37/50, 74 %) or lenvatinib (7/50, 14 %), while 75 patients had died without second-line treatment. Median PFS (progression-free survival) for first-line therapy was 6.1 months (95 %-CI 4.7-8.5), median OS (overall survival) was 9.7 (7.6-11.6) months.

Conclusion ATZ + BEV was quickly adopted as the new standard of care for aHCC. Patients in real-world are older and have more comorbidities compared to clinical trials which is reflected in the outcomes data.

P4.06 Expression of target-independent membrane-associated T-cell engagers (MATEs) provides oncolytic adenoviruses with effective T-cell activation capabilities and unrestrained applicability

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Oncolytic viruses (OV) are promising tools for immunotherapy of a broad range of tumors and highly effective when armed by expression of additional immunostimulatory mediators. To realize potent T-cell stimulator proteins suitable for expression by oncolytic adenoviruses without restricting their broad-range applicability, we established soluble, target-independent, membrane-binding T-cell Engagers (MATEs). MATEs contain a αCD3scFv linked to the protein transduction domain of HIV-Tat to achieve efficient, but non-selective cell binding. In vitro, MATEs effectively activated murine T-cells as shown by CD25, CD69, Ki67 and IFNγ. MATEs improved direct killing of murine MC38 colon carcinoma cells, as a standard cell line for immunotherapeutic investigations. In vivo, a novel chimeric Adenovirus (Ad5/11) was used for MATE-expression, genetically linked to E1B via an IRES element for low expression levels. After i.t. injection in MC38 tumors in C57BL/6 mice, intratumoral T cells were significantly activated,but no activation was detected in tumor draining lymphnodes suggesting that T-cell activation was limited to tumor tissue and confirming safety of this target-independent approach. Antitumoral immunity reduced tumor growth and prolonged survival accompanied by improved infiltration of CD8 T-cells and CD8/CD4 ratio. Luminex assays showed altered levels of intratumoral cytokines, including IL-2 and IL-6. MATE-expressing virotherapy increased therapeutic efficacy of αPD-1 checkpoint blockade, accompanied by induction of tumor-specific T-cells. Multiplex IHC suggested formation of T-cell clusters and an improved T-cell/tumor cell proximity. In summary, non-target selective MATEs for T-cell activation are a well suited for arming OVs but maintaining their applicability in a broad range of tumors.

P4.07 Imaging Mass Cytometry as a method for improved diagnostics in patients with CUP syndrome and primary manifestation in the liver

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Background The diagnostic algorithm for immunohistochemistry in patients presenting with metastatic cancer of unknown primary in the liver (CUP syndrome) remains a challenging and time-consuming process of several consecutively stained tumor markers. We created a panel for identification of tumor origin in one single staining process using Imaging Mass Cytometry.

Methods Formalin-fixed paraffin-embedded (FFPE) samples from liver metastasis tissue of 41 patients presenting with different entities of CUP syndrome were stained using a 43-marker panel addressing the most frequent tumor origins in CUP syndrome as well as identifying different subgroups of potentially targetable immune cell populations and receptors for targeted therapy. All 43 markers were stained in one single staining process and images were acquired using Imaging Mass Cytometry.

Results We were able to successfully establish a unique panel identifying origins of liver metastases e.g. mamma carcinoma, different tumors of intestinal origin or melanoma. Identification of most tumor origins was possible using the information of the acquired Imaging Mass Cytometry images. We also gained further information regarding immune cell populations and immune receptors of the different entities.

Conclusion Imaging Mass Cytometry presented itself as a promising approach for an improved and accelerated diagnostic algorithm in patients presenting with CUP syndrome regarding the identification of tumor origin.

P4.08 Next Generation Sequencing of bile to support diagnosis and decision making in patients with malignant biliary strictures

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Background Cholangiocarcinomas are rare tumors with an increasing incidence, but diagnosis is often challenging, especially in patients at risk (e.g. PSC patients) and extrahepatic cholangiocarcinomas. Currently, standard workup includes modern imaging modalities and ERC/cholangioscopy–guided biopsies, but especially the detection of early malignant disease remains suboptimal. Next generation sequencing from liquid biopsies offers additional options for the diagnosis of cancer, and molecular diagnostics from bile, collected during routine ERC procedures, may complement established diagnostic approaches and support clinical decision making. We aim to integrate bile-based NGS into our clinical workup for patients at risk of cholangiocarcinoma and with suspicious biliary strictures.

Materials&Methods Bile was collected from patients during ERC procedures, centrifuged and stored at -80 °C until further usage. In parallel, blood was collected in Streck tubes. Cell-free DNA was isolated using the QIAamp Circulating Nucleic Acid Kit. DNA concentration was quantified using Qubit dsDNA High-Sensitive Assay kit on the Qubit fluorometer. DNA was analyzed with the Oncomine Comprehensive Assay v3.

Results So far, bile samples from 70 patients were collected, including 13 cholangiocarcinoma patients and 28 patients with PSC without an established malignant diagnosis. cell-free DNA concentration was significantly higher in bile than in blood (94.80 [364.0]ng DNA/µl vs. 0.42 [0.88]ng DNA/µl (median [IQR])). Pathogenic mutations, including KRAS mutations, were found in patients with the established diagnosis of CCA, as well as in PSC patients with suspicious findings on imaging and ERC.

Discussion Our data support further investigation of the potential of complementary bile-based NGS diagnostics for early diagnosis of cholangiocarcinomas.

P4.09 Identification of blood based biomarkers for hepatocellular carcinoma screening

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Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide with rising incidence. It is the third leading cause of cancer-related deaths, in part because of inadequate early detection strategies. Current recommendations for screening include biannual ultrasonography with or without alpha -fetoprotein, but has limited sensitivity and accuracy. A blood based non-invasive biomarker could aid in overcoming the deficiencies of the present methods. With the advancements in transcriptomic techniques, the in-depth profiling of the immune cells in the peripheral blood of HCC patients has become possible. From this study, we wish to determine the transcriptional footprint of immune cells in HCC to facilitate the detection of prognostic biomarkers in patients. We performed single cell RNA sequencing on the peripheral blood samples of 9 patients with HCC. Blood sampling was performed prior to and post either surgical resection or ablation of the tumors. The differentially expressed genes from the analysis will be studied for biomarker identification using flow cytometry. From the transcriptomics data, we observed the highest changes in the differentially expressed genes (DEGs) in the monocyte subpopulations in comparison to the other immune cells. Interestingly the DEG profile was contrasting in the patients that relapsed post-surgery in comparison to the non-relapsed patients. Gene set enrichment analysis suggested an upregulation in the type-1 and type-3 interferon response genes in the non-relapsed patients. Our results suggest the presence of an immune signature of monocytes in HCC patients which can be used as a potential prognostic biomarker for prediction of recurrence risk.

P4.10 In situ detection of ablation area extension and local tumor necrosis using self-constructed fiberoptic system

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Introduction The maximum effect of the local ablation (radiofrequency and microwave therapy) of liver tumors is achieved when the ablation region completely encloses the tumor area. In the present study, the self-constructed system based on fiberoptic detection of tissue autofluorescence was used for identification of local extension of ablation area and of chemotherapy-induced local tumor necrosis.

Methods Thermoablation area of different size was produced ex vivo in porcine liver using clinical high-frequency surgery or microwave ablation devices. Tumor inoculation in chorio-allantoic membrane was used as model for solid human tumor growth. The autofluorescence in specific spectrum (470-480ex/520-530em) was measured in necrotic and vital tissue using fluorescence microscopy and self-constructed fiberoptic fluorescence detection system. Fiberoptic detection was also used to detect the real-time in situ changes of the autofluorescence during thermoablation or tumor chemotherapy (5-FU).

Results As indicated by both fluorescence microscopy and fiberoptic detection, thermoablation and chemotherapy produced necrotic tissue that was characterized by highly increased autofluorescence in comparison with vital tissue. Using increase of the autofluorescence, the self-constructed fiberoptic system enabled the sensitive in situ detection of exact position of the necrotic margin during ablation procedure. The procedure was realizable in the real-time mode and was applicable for any size of ablation area. Furthermore, fiberoptic detection enabled the exact in situ identification of the chemotherapy-induced tumor necrosis.

Conclusion Autofluorescence-based fiberoptic detection represents the sensitive tool for exact in situ detection of necrotic tissue and could significantly improve results of local ablative tumor therapy.

P4.11 Development of a model to analyze the relevance of MHC class II epitopes for hepatocellular carcinoma immunity

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The role of CD4 T cells in tumor immunity is not yet understood, as CD4 T cells recognize MHC class II epitopes expressed on antigen-presenting cells but not on tumor cells. Still, they send critical signals to dendritic cells, which then upregulate the expression of MHC class I molecules, costimulatory molecules, and secretion of IL-12 and IL-15, thereby driving CD8 T cells response for lysis of tumor cells.

The overall goal of our study is to determine the importance of CD4 T cells in adaptive immune responses and immunotherapy against HCC.

Mice were inoculated with HCC cells with different MHC class II expressions, containing defined CD8 and CD4 T cell neoepitopes, and the specific T cell immune responses were analyzed. Early evaluations indicate increased CD8 T cell immune response in MHC-II positive tumors and tumors with CD4 T cell neoepitopes.

In a subcutaneous cancer model we will use therapeutic T cell vaccinations in order analyze the anti-tumoral T cell responses by flow cytometry and to evaluate the relevance of CD4 epitope and MHCII expression in cancer cells.

Our study will bring us closer to understanding the importance of CD4 T cell help in HCC.

P4.12 Characterization of the cellular expression pattern of bile acid receptors in intrahepatic cholangiocellular carcinoma

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Cholangiocellular carcinoma (CCA) arises from the epithelial cells of the bile ducts and can be classified according to its location along the biliary tree as intrahepatic (iCCA), perihilar or distal. Each subtype has a distinct epidemiology, biology, prognosis, and strategy for clinical management.

Pathogenesis remains elusive, and only recently, bile acids (BA) as potent signalling molecules have been linked to CCA development. Nevertheless, the spatial expression pattern of the different bile acid receptors in human CCA and adjacent non-tumour tissue is unknown. However, this knowledge could be the cornerstone of potential new bile acid receptor-associated therapeutic strategies in CCA.

In our project, we want to study the tumour microenvironment of iCCA and adjacent non-tumorous tissue with special regard to the cellular expression pattern of bile acid receptors by using Multiplex immunostaining. This technique is a sequential immunostaining protocol consisting of repetitive cycles of staining and chemically induced antibody stripping followed by image processing, allowing the study of up to 10-20 targets simultaneously on one Formalin-fixed paraffin embedded (FFPE) tissue section. Ultimately, we aim to correlate our experimental findings with clinical parameters such as therapy response or survival of the patients. First results indicate an overexpression of TGR5 (GPBAR1), a G-protein-coupled receptor responsive to BA, in CCA tissue.

P4.13 The role of A20 as a master switch for the regulation of multiple distinct cell death pathways in hepatocarcinogenesis

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TNFAIP3 is an NF-κB target gene whose encoded protein A20 terminates the activity of NF κB in response to TNF and microbial products such as lipopolysaccharides (LPS) in a negative feedback mechanism. Furthermore, it is also known as an inhibitor of TNF-induced apoptosis, while its molecular and functional role in the regulation of various programmed cell death (PCD) mechanisms and the development of hepatocellular carcinoma (HCC) has not yet been studied in depth, especially in relation to RIPK1 as a regulator of apoptosis and necroptosis.

Floxed A20 mice were crossed with mice expressing cre-recombinase under a liver paren-chymal cell specific (LPC) albumin promoter with alpha-fetoprotein enhancer. The resulting A20LPC-KO animals were further crossed with RIPK1LPC-KO mice. LPS injection was used as a model to simulate acute liver injury. Liver damage was assessed using serum transaminase level measurement and immunohistochemical stainings.

In the livers of A20LPC-KO mice, simultaneous apoptotic and necrotic damage could be ob-served after LPS injection. The additional deletion of RIPK1in the A20-deficient mice was related to an impressive spontaneous phenotype. This manifests itself in an altered hepatocyte architecture as early as 6 weeks of age, which develops into massive liver tumours by 50 weeks of age. Interestingly, HCC development in these mice was associated with NF-κB hyperactivation.

Our data suggest that A20 has a protective function in the liver due to inhibition of apoptosis and necroptosis. Furthermore, these findings implicate that A20 might be a crucial interaction partner of RIPK1 linking inflammation and PCD thus controlling HCC.

P4.14 Transcriptional profiling of tumor-specific CD8 T-cells shows contribution of TIGIT to T-cell exhaustion in liver cancer

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Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death and the fifth most common kind of cancer worldwide. Anti-cancer immune responses are often hampered by upregulation of co-inhibitory receptors on the surface of CD8 T-cells accompanied by reduced T-cell functionality, described as T-cell-exhaustion. We have utilized the Sleeping beauty" (SB)/transposon system for the development of an autochtonous HCC mouse model. Using adoptive T-cell transfer allowed us in-depth phenotyping of tumor-

specific CD8 T-cells and we could demonstrate upregulation of co-inhibitory receptors, including PD-1 and TIM-3 on the T-cell surface. The tumor-specific CD8 T-cells also showed a reduced IFNγ expression, indicating T-cell exhaustion. We have performed the first whole transcriptome microarray analysis of tumor-specific CD8 T-cells in a murine autochthonous liver cancer model, that allowed us to compare the mRNA profiles of naive, functional effector and exhausted tumor-specific CD8 T-cells. Transcriptomic data was analyzed for identification of candidate genes and pathways that play a role in T-cell exhaustion. Particularly, the substantial upregulation of TIGIT suggested the involvement of this inhibitory T-cell receptor in T-cell exhaustion in liver cancer. Utilization of immune checkpoint-blockade against TIGIT in combination with PD-1 inhibition prolonged survival of tumor-bearing mice, compared to anti-PD-1-monotherapy. We could further verify the expression of TIGIT on tumor-infiltrating CD8 T-cells in HCC-patients. Our results suggest that TIGIT is involved in the appearance of T-cell exhaustion in human HCC and presents a potential target for combination treatment by immune checkpoint blockade.

P4.15 Shift in cellular crosstalk reveals the immunosuppressive environment in cholangiocarcinoma patients

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DOI 10.1055/s-0043-1777599

Introduction Cholangiocarcinoma (CCA) is a very deadly disease and treatment options, besides surgery, are limited. This cancer entity is characterized by abundant stroma and here crosstalk between fibroblasts, immune cells and cancer cells take place. The TME plays an important role in the poor response rate to therapeutics, but not much is known about the mechanisms behind this.
Material and Methods Here we used cytometry by time of flight (CyTOF) to phenotype T cells from 16 human CCA tumor samples as well as matched adjacent liver tissue. In addition, we used single nucleus RNA sequencing of 7 CCA samples to examine crosstalk between identified cell populations.
Results We show a high level of exhaustion in effector T cells in the tumor niche as well as high levels of activated regulator T cells (Tregs), while CD8 effector memory (EM) and tissue-resident effector memory (TRM) cells fail to invade the tumor. We identified a shift in crosstalk between the stromal and hepatic stellate cell (SCHSC) with cholangiocytes, mainly present in the poor patient outcome group. The cell communication specific for the poor patient group is enhanced between the cholangiocytes and macrophages/myeloid cells. TGFβ signaling as well as the Gas6/Axl pathway were involved.
Conclusion This study shows a suppressive tumor immune environment characterized by T cell exhaustion and exclusion of cytotoxic T cells. Furthermore, we shed light on the etiology of the immune suppressive TME in CCA and uncover involved pathways that provide better understanding of the resistance to therapeutical agents.

P4.16 Nerve fibers in the tumor microenvironment as a novel biomarker in perihilar cholangiocarcinoma

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Introduction Perihilar cholangiocarcinoma (pCCA) is a biliary tract cancer with a dismal prognosis, with surgery being the only chance of cure. A characteristic aggressive biological feature of pCCA is perineural growth which is defined by the invasion of cancers cells into nerve fibers. Recently, nerve fiber density (NFD) was linked to oncological outcomes in various malignancies, however, its prognostic role in pCCA remains to be elucidated.
Material and Methods Data of 101 pCCA patients who underwent curative intent surgery between 2010 and 2019 were included in this study. NFD counts were performed based on expression of PGP9.5 immunohistochemistry. Extensive group comparisons between patients with high and low NFD were carried out and the association of cancer-specific survival (CSS), recurrence-free survival (RFS) with NFD and other clinico-pathological characteristics were assessed using univariate and multivariable cox regression models.
Results Patients with high NFD showed a median CSS of 90 months (95 % CI: 48-132, 3-year-CSS = 77 %, 5-year-CSS = 72 %) compared to 33 months (95 % CI: 19-47, 3-year-CSS = 46 %, 5-year-CSS = 32 %) in patients with low NFD (p = 0.006 log rank). Patients with high NFD and negative lymph nodes, showed a median CSS of 90 months (3-year-CSS = 88 %, 5-year-CSS = 80 %), and patients with both positive lymph nodes and low NFD a median CSS of 24 months (3-year-CSS = 26 %, 5-year-CSS = 16 %, p = 0.001 log rank).
Conclusion NFD has been identified as an important novel prognostic biomarker in pCCA patients. NFD alone and in combination with nodal status, allow to stratify pCCA patients based on their risk for inferior oncological outcomes after curative-intent surgery.

P4.17 High-dimensional spatial profiling of the hepatocellular carcinoma tumor microenvironment reveals spatial immune types informing immune checkpoint inhibitor therapy response

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Introduction Hepatocellular carcinomas (HCC) can be grouped into distinct immune subtypes according to their molecular profiles. However, clinical translation, especially in the context of immune checkpoint inhibitor (ICI) therapy, is lacking, which may be due to the negligence of spatial context. Thus, we set out to characterize the HCC TME on a spatially resolved and high-dimensional level using imaging mass cytometry (IMC).
Methods We performed IMC analysis using a 41-marker panel on FFPE sections of 54 HCC patients with regions of interested spanning the tumor, interface and adjacent liver. After channel normalization and cell segmentation, we clustered single-cells and identified immune neighborhoods based on spatial immune cell interactions. We reapplied the same workflow to a validation cohort of 42 HCC patients that received ICI based therapies after tumor biopsy or resection.
Results We identified distinct tumor immune microenvironments based on the quantity and composition of infiltrating immune cells. Specifically we distinguished three major subtypes: immune-depleted, immune-intermediate and immune-enriched. We observed a differential organization of the TME and the interface area between spatial immune types. Unsupervised neighborhood detection identified three immune neighborhoods that correlated with spatial immune types. Immune types were not clearly connected to known HCC etiologies or frequent mutations but correlated with progression-free survival under ICI based therapies.
Conclusion In sum, our in-depth spatial analysis successfully captured the immune heterogeneity of HCC patients. Spatial immune types may be reflective of different immune evasive strategies of the corresponding tumor and represent a potential novel biomarker for ICI based therapies.

P4.18 Non-invasive high throughput monitoring of exon-specific p53 isoform expression identifies new promising therapeutics for cancer patients with altered p53 status

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Background Studies aiming at the targeted activation of tumor-suppressive p53 isoforms have revealed promising and novel therapeutic approaches. Besides the pro-apoptotic isoforms full-length (FL)p53 and Δ40p53, Δ133p53 and Δ160p53 act as negative regulators inducing anti-apoptotic effects. We established a novel exon-specific and luciferase-based reporter system (EXISERS) that allows for multiplexed quantification and differentiation of target-protein isoforms in real-time and living cells. Its high-throughput capability allows for systematic drug-related studies on p53 isoform expression.

Method We stably integrated three exon-specific isoform expression reporter systems (EXSISERS) into TP53 HCT116 cells via CRISPR/Cas9. This allows for real-time detection of the expressional change of the three p53 isoforms-groups FLp53, Δ40p53, and Δ133 + Δ160p53. Furthermore, we investigated the impact of 4,863 anti-cancer compounds (MCE, HY-L025) on differential expression profiles of the respective p53 isoforms in a high-throughput manner. Additionally, we assessed the efficacy and tumor-specificity of potential drug candidates on colonic cancer organoids.

Results We demonstrate that EXSISERS offers advantages over conventional protein detection methods (Western blot and mass spectrometry). Utilizing high-throughput screening with EXSISERS, we investigated differential p53 isoform expression in response to 4,863 small molecules. Here, we identified new compounds efficiently inducing cell death by highly upregulating tumor-suppressive FLp53 and Δ40p53 while efficiently downregulating pro-survival Δ133 + Δ160p53.

Conclusion EXSISERS is a new reporter system applicable in high-throughput screening to quantitatively measure p53 protein isoforms in real time. Our versatile system allows for the identification of anti-tumor therapeutics that efficiently induce pro-apoptotic p53 isoforms, enabling targeted cell death in cancer cells of various tumor entities.

P4.19 Cancer-associated fibroblasts specific gene silencing for anti-stromal therapy in primary liver cancer using novel anti-MFAP-5 siRNA-loaded polymeric nanoparticles

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Background Cancer associated fibroblasts (CAF) support tumor growth and metastasis in the tumor microenvironment (TME) and are therefore promising target cells for anti-stromal therapy in hepatocellular carcinoma (HCC) [Kaps, Schuppan; Cells 2020].

Results Nanoparticles (NP) have been designed utilizing the triblock copolymer polysarcosine-b-poly(γ-benzyl glutamic acid)-b-polylysine, which enables co-loading of siRNA and desloratadine (DES), a small molecule to trigger endosomal release. For the HCC model, healthy B6 mice were intrasplenically injected with 500.000 HCC cells to develop macroscopic tumor lesions exclusively in their livers after 28 days For in vivo anti-stromal therapy, tumor mice (n = 5) received three intravenous injections of NP loaded with anti-MFAP-5 siRNA (corresponding to 0.5 or 1 mg/kg siRNA) in week four, while controls received equal concentrations of NP loaded with non-targeting scrambled siRNA (scsiRNA). Liver weights of mice treated with anti-MFAP-5 siRNA were significantly ($^*p < 0.05$) lower compared to mice treated with encapsulated scsiRNA, indicating less hepatic tumor burden. The treatment was well tolerated by the mice and serum parameters for liver- and nephrotoxicity were in the normal range. Histological analysis of liver sections revealed that markers of tumor vascularization (CD34 and CD105) were down regulated in the TME, suggesting an anti-angiogenic effect of the siRNA treatment, which was supported by the transcriptome analysis of whole liver tissue.

Conclusions Liver targeting NP loaded with siRNA induced a gene specific knockdown of CAF derived MFAP-5 and demonstrated a convincing antitumor effect by interference with angiogenesis in the TME of HCC

P4.20 Oncogene-induced long non-coding RNA (lncRNA) signatures in liver cancer

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Robust and sensitive biomarkers are essential for patient stratification and personalized treatment.

Novel biomarkers are identified by comparing samples from healthy and diseased individuals. However, this cannot provide a comprehensive picture of relevant alterations in tumor cells, such as the activation of therapy-relevant pathways or transcription factors.

In this study, we integrated experimental data from next-generation sequencing (NGS), ChIP-seq results, and clinical data from liver cancer patients to identify signaling pathway- and transcription factor-specific long non-coding RNA (lncRNA) signatures. Using this approach, we found that the lncRNAs CYTOR, MIR4435-2HG, SNHG1, and SNHG17 are transcriptionally regulated by the oncogenic Hippo pathway effector yes-associated protein (YAP). These individual lncRNAs functionally support tumor growth in different cell lines. Notably, a gene signature consisting of these four lncRNAs in the serum of a subcutaneous transplantation model and hepatocellular cancer (HCC) patients is a robust predictor of YAP activation in cancer cells. We conducted follow-up experiments to investigate whether lncRNA signatures can be used as biomarkers for other transcriptional complexes. Indeed, NGS analysis revealed alternative lncRNA signatures in HCC cells after gene-specific inhibition of the WNT pathway effectors β-catenin and transcription factor 4 (TCF4, TCF7L2). Lastly, we demonstrate that the presence of lncRNA signatures defines the response of YAP-directed pharmacological inhibition in vitro.

In summary, transcriptionally regulated lncRNA signatures could be novel and robust serum biomarkers for signaling pathways or oncogene activity in liver cancer. Additionally, we demonstrate the potential of lncRNA signatures as predictors for treatment response.

P4.21 Überlebensdatenanalyse von Patienten mit hepatozellulären Karzinoms (HCC) unter First-Line Therapie mit Atezolizumab/Bevacizumab (AB) in Abhängigkeit der Leberfunktion – Analyse über 4 Jahre (01/2019–03/2023) eines universitären Zentrums

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Hintergrund Die Leberfunktion ist ein prognostischer Faktor bei Patienten mit HCC im Stadium BCLC B/C. Es existiert unzureichend wissenschaftliche Evidenz über die Effektivität von Atezolizumab/Bevacizumab (AB) bei eingeschränkter Leberfunktion und Leberzirrhose Child-Pugh (CP) B.

Ziel Einfluss der Leberfunktion auf das Überleben sowie die Therapiesequenz.

Methoden In eine retrospektive Analyse (01/2019-03/2023) wurden 49 Patienten mit HCC und Erstlinientherapie mit AB eingeschlossen und die Leberfunktion mittels CP-Score und ALBI Score objektiviert.

Ergebnisse Bei Therapiebeginn lag bei 44.9 % der Patienten ein HCC im Stadium BCLC B (n = 22) vor, bei 53.1 % ein HCC im Stadium BCLC C (n = 26). 51 % der Patienten hatten Ösophagusvarizen. 77.6 % (n = 38) der Patienten zeigten initial eine Leberzirrhose im Stadium CP A, 22.4 % (n = 11) im Stadium CP B. Nur 36.7 % der Patienten (n = 18) wurden mit einer Zweitlinientherapie behandelt, davon alle mit initialem CP A Stadium. Das Erreichen der Zweitlinientherapie war statistisch signifikant mit einer besseren Leberfunktion assoziiert (p = 0.004). Bei Patienten CP A betrug das Gesamtüberleben (OS) 412 Tagen (95 %CI:371-453), bei CP B betrug das OS 161 Tage (95 %CI:62-260). Das OS war statistisch signifikant mit einer verbesserten Leberfunktion assoziiert (p = 0.013; Spearman p = -0,458). Patienten mit ALBI-Grad1 erreichten ein medianes OS von 451 Tagen (95 %CI:281-621), bei Patienten mit ALBI-Grad2 war das mediane OS mit 240 Tagen (95 %CI:142-338) statistisch signifikant reduziert (p = 0.02).

Schlussfolgerung Bei Patienten mit eingeschränkter Leberfunktion ist das Gesamtüberleben signifikant reduziert. Nur ein geringer Anteil der Patienten erhält nach AB eine Zweitlinientherapie.

P4.22 Characterization of selinexor response/resistance in hepatocellular carcinoma

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Background and Aims The nuclear transport system is intrinsically linked to tumor-relevant signaling cascades and selective inhibitors of nuclear export represent a promising approach for cancer treatment. The exportin-1 (XPO-1) inhibitor selinexor is already approved for treating hematological neoplasms, whereas its efficacy in solid tumors including liver cancer is poorly understood. In this study we aim to identify candidates potentially involved in selinexor response/resistance.

Method A variety of human (HepG2, Hep3B, HLE and HLF) liver cancer lines were treated with selinexor under several conditions. The cells were then analyzed via qPCR, MTT viability assays and LC-MS/MS-based proteomics. Potential response- or resistance-related candidates were further investigated via silencing (siRNA) or overexpression (cDNA) in vitro.

Results HepG2 and Hep3B responded well to selinexor treatment (0.25 µM, 72 h) showing a residual viability of 25 %, whereas HLE and HLF remained largely unaffected. Proteomic analyses revealed significantly higher abundance of Glutathione S-transferase P (GSTP1), Myoferlin (MYOF) and Profilin-1 (PFN1) among others in the selinexor-treated non-responding group. These candidates were previously linked to exosome formation and cytoskeleton remodeling as well as drug resistance. Furthermore, we detected a dose-dependent increase of programmed death ligand 1 (PD-L1) mRNA expression (up to ~11-fold) upon selinexor treatment.

Conclusion Our data suggest that selinexor resistance in HCC is related to proteins involved in exosome formation and cytoskeleton remodeling. In addition, selinexor directly or indirectly affects PD-L1 expression in liver cancer with potential relevance for (combined) immune checkpoint therapy.

P4.23 Genetic variation in angiotensin II type 1 receptor is linked to lipid levels and hepatic steatosis in alcohol-associated liver disease, but not to complications of liver disease

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Background Genetic predisposition modulates development of cirrhosis and hepatocellular carcinoma (HCC) in patients with high alcohol-associated liver disease . For non-alcoholic fatty liver disease, genetic polymorphisms in angiotensin II type 1 receptor (AGTR1) have been described as risk factor.

Methods We analysed healthy controls, Caucasian patients with alcohol-associated cirrhosis without (n = 238) and with (n = 339) HCC, HCV-infected cirrhotic patients with and without HCC (n = 263) for association with the polymorphisms rs3772622 and rs2276736 in AGTR1.

Results Rs2276736 in AGTR1 was linked to both low density lipoprotein (LDL) cholesterol levels and hepatic steatosis in patients with alcohol-associated liver disease. Distribution of genotypes for both rs3772622 (and rs2276736) in AGTR1 were comparable between healthy controls, patients with cirrhosis and with HCC. In detail, minor allele frequencies were 32 % (44 %) in healthy controls, 35 %/34 % (46 %/45 %) in alcohol-associated liver disease without/with HCC and 31 %/38 % (43 %/39 %) in HCV cirrhosis and HCV HCC, respectively. The genotype of the most important genetic risk factor for fatty liver disease, PNPLA3 I148M, did not interact with the AGTR1 polymorphisms.

Conclusion Genetic variation in AGTR1, although associated to blood lipid levels and hepatic steatosis, is not a risk factor for alcohol-associated cirrhosis or HCC.

P4.24 Circulating miRNA with predictive value in patients with hepatocellular carcinoma treated with atezolizumab/bevacizumab

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Background Resistance to immune checkpoint inhibitor (ICI) therapies is common among patients with hepatocellular carcinoma (HCC). MicroRNA (miRNA), small non-coding RNA transcripts, are involved in post-transcriptional gene silencing and can influence immune checkpoint expression of cancer and immune cells. This study aims to assess the role of circulating miRNA in ICI resistance in HCC.

Methods Patients with HCC treated with atezolizumab/bevacizumab and available baseline blood samples were screened and classified according to their objective response in the first staging into early responders and progressors. Global profiling of around 1000 circulating miRNA in the blood was conducted in a discovery cohort (n = 12) and differentially expressed (DE) candidate miRNA were identified (p < 0.05). DE miRNA were subjected to miRNA enrichment analysis.

Results We identified miRNA with reliable differential expression between early responders and progressors treated with atezolizumab/bevacizumab. Baseline characteristics among both groups were similar. miRNA enrichment analysis revealed that differentially expressed miRNA were involved in regula-

ting T-cell function, suggesting that the identified miRNA could be reflective of ICI-resistance mechanisms in HCC.

Conclusion Circulating miRNA may predict treatment resistance in HCC patients treated with atezolizumab/bevacizumab. To validate this hypothesis, the identified miRNA will be investigated in an expanded cohort.

P4.25 Repurposing passenger amplifications for specific therapeutic targeting of liver and other solid cancers

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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.

P4.26 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, SNU-449) 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 Y4R, si-RNA-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. Senescence analysis were carried out by measuring β-galactosidase activity.

Results Y4R and PP expression levels were strongly upregulated in HCC in vitro and in patient tissues. PP induced both chemoattractive and chemokinetic migration. Knockdown of Y4R effectively rescued this migration, resulting in decreased clonogenicity, reduced proliferation, and the induction of senescence.

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

P4.27 Revisiting Non-Invasive Diagnostic Approach for Hepatocellular Carcinoma in Clinical Practice

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Background Current guidelines emphasize the use of CT or MRI for hepatocellular carcinoma (HCC) diagnosis and restricting biopsies. However, hepatocellular-cholangiocarcinoma (cHCC-CC), a variant displaying features of both HCC and cholangiocellular carcinoma (CCC), presents mixed radiological traits. Unlike HCC, platinum drugs are the most promising primary treatment for unresectable cHCC-CC. Our study assesses imaging techniques' efficacy in distinguishing HCC, CCC, and cHCC-CC, emphasizing its clinical importance.

Methods We analyzed 68 MRI scans (30 HCC, 23 CCC, 15 cHCC-CC) from patients diagnosed between 2010 and 2020. The diagnoses were histologically confirmed within four weeks of the MRI scans. Seven radiologists, including experts in abdominal (AIEs) and non-abdominal imaging (NIEs) or trainees from Asia, Europe, North America, and South America, independently evaluated the scans, analyzing diverse MRI features to establish differential diagnoses for HCC, CCC, and cHCC-CC.

Results The AIEs demonstrated superior proficiency in utilizing MRI images for diagnosing HCC (70 %-100 %) and CCC (73.9 %-91.3 %), outperforming NIEs/trainees significantly (all p values < 0.01) with accuracy rates of 26.7 %-66.7 % for HCC and 21.7 %-60.9 % for CCC. However, their ability to distinguish cHCC-CC (6.7 %-53.3 %) was limited and comparable to NIEs/trainees (26.7 %-46.7 %). AIEs exhibited greater consistency in MRI feature assessment for HCC and CCC compared to cHCC-CC. Moreover, there was no significant intercontinental variability in liver cancer diagnosis by AIEs.

Conclusion MRI effectively differentiates HCC and CCC, particularly with expert interpretation. However, accurate cHCC-CC detection remains challenging for all participating radiologists. Hence, liver biopsy remains crucial for precise diagnosis and treatment strategy selection.

P4.28 Spatially mapping complex genotype-to-phenotype relations in an autochthonous liver cancer mouse model

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Intratumoral genetic heterogeneity and tumor-stroma interactions are emerging topics in cancer research. However, the lack of experimental strategies has so far precluded systematic and unbiased analyses of how heterogenous

complex genetic alterations can affect tumor development and the tumor micro-environment in a native context.
We have devised a novel approach for conducting spatially-resolved in vivo functional genomics and apply this method to a newly developed autochthonous liver tumor heterogeneity mouse model.
We successfully demonstrate that a single spatial transcriptomics readout platform can be used to study complex genotype-to-phenotype relationships among over 200 cancer clones coexisting within the native tissue environment of a single mouse liver.
Our proof-of-concept study reveals limitations and opportunities of our current approach.

P4.29 From genotype to phenotype: how IDH1 mutations alter the landscape of intrahepatic cholangiocarcinoma

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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 the introduction of genetic elements directly into the liver. Our results revealed that IDH1 mutations combined with other iCCA-driving oncogenic events shorten the 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 an altered abundance of stromal and immune cells in the tumor microenvironment. Further, to identify key players contributing to 2-HG-driven phenotype, we apply mass spectrometry analyses of the extracellular matrix from liver cancer tissue.
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 key elements that contribute to the 2-HG-driven phenotype, providing direction for future therapeutic development in the context of IDH1-mutant iCCA.

P4.30 Discovering synthetic lethal interactions caused by chromosomal deletions

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In this project, we aim to identify synthetic lethal genes associated with chromosomal deletions. As a blueprint, we will use chromosome 1p, as the deletion of it is an early event, e.g. in liver cancer. First, in silico data mining will be applied to identify genes of interest. These genes will be subsequently validated using reverse genetic screens. For this, libraries of sgRNAs and/or shRNAs will be generated targeting the identified genes as well as respective controls. After cloning into expression plasmids, these will be lentivirally infected into human cancer cell lines, preferably of the liver, either harboring chromosome 1p deletion or cell lines without 1p deletion. After narrowing down candidate genes, individual gene knockouts will undergo several functional tests comparing their impact, e.g. on proliferation between the knockout cell lines with and without 1p deletion. If these approaches were successful, the next step would be to investigate the impact of their deletion in vivo. By inducing respective chromosomal deletions in tumors coupled with simultaneous depletion of respective candidate genes via RNA interference, that will allow to investigate the consequences on tumor development and maintenance. In summary, these experiments will help to identify synthetic lethal interactions of chromosomal deletions, which are hallmarks of cancer genomes.

P4.31 Prediction of response to atezolizumab/bevacizumab in advanced hepatocellular carcinoma through radiomic features in pretreatment MRI

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The advent of Atezolizumab/Bevacizumab as the first-line systemic therapy for advanced HCC marks a significant advancement. However, the need for biomarkers to prognosticate drug response persists. Radiomics, a method involving the conversion of medical images into high-dimensional quantitative features, holds promise for yielding novel biomarkers. This study aims to identify new biomarkers, derived not only from clinical data but also from pretreatment contrast-enhanced MRI via radiomics feature extraction, aiming to forecast therapeutic response.
A retrospective analysis was conducted on patients with advanced HCC treated with Atezolizumab/Bevacizumab across six centers in Germany and Austria. Response assessment mandated an initial MRI and a subsequent radiological evaluation (CT or MRI) after three months of treatment. The cohort was stratified into two groups based on mRECIST criteria: controlled disease (encompassing complete response, partial response, or stable disease) and disease progression. The liver and all tumoral lesions were segmented in the initial MRI, from which radiomics features were extracted. The relevant radiomics features were then employed to predict treatment response and overall survival.
Of the 104 patients, 70 exhibited controlled disease while 34 faced disease progression. Factors such as hepatitis B virus etiology, ascitis presence, prior systemic therapy, elevated C-reactive protein levels, and metastatic disease correlated with progression. The progressive disease group demonstrated significantly diminished progression-free survival (81.5 days vs 298.5 days, $p = 0.001$) and overall survival (150.0 days vs 568.5 days, $p = 0.001$). By combining clinical parameters with relevant radiomics features, a predictive model for disease progression was developed.

P4.32 Prediction of YAP/TAZ activation in liver cancer patients based on gene expression and clinical data

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The Hippo pathway, governed by the effector proteins YAP and TAZ, plays a pivotal role in regulating cell proliferation and maintaining tissue homeostasis. Dysregulation of YAP and TAZ has been implicated in several cancers, including Hepatocellular carcinoma (HCC). While pharmaceutical companies are actively developing YAP/TAZ inhibitors, there is currently no means to predict suitable patients for targeted therapy.
To address this, we used gene expression and clinical data of 370 HCC patients obtained from the cancer genome atlas consortium. Employing established

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YAP/TAZ signatures, we classified patients into distinct groups, reflecting varying degrees of YAP/TAZ activation. High signature expression was associated with poor overall survival, p53 mutations, female gender, and lower tumor grading. Using clinical parameters, we developed a predictive model to discern YAP/TAZ activation and identified a set of crucial genes for patient stratification. Moreover, the subclustering of patients was recapitulated in mouse models using hydrodynamic tail vein injection of different oncogenes in combination with either YAP or TAZ.

In conclusion, the results from our analysis may help to identify patients with YAP/TAZ activation based on clinical parameters and a minimal panel of genes. With this, patients could be selected for YAP/TAZ targeted therapies.

P4.33 KIF23 regulation by miR-107 controls replicative tumor cell fitness in mouse and human hepatocellular carcinoma

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Background In hepatocellular carcinoma, there is a lack of successful translation of experimental targets identified in mouse models to human patients. In this study, we used a comprehensive transcriptomic approach in mice to identify novel potential targets for therapeutic intervention in humans.

Methods We analyzed genome-wide miRNA and mRNA expression data in three pathogenically distinct mouse models of liver cancer. Effects of target genes on hepatoma cell fitness were evaluated by proliferation, survival and motility assays. TCGA and GEO databases, in combination with TMAs were used to assess the impact of mouse targets on human HCC prognosis. Finally, the functional effects of the identified targets on tumorigenesis were tested in HDTVi-based preclinical HCC models in vivo.

Results The expression of miR-107 was found to be significantly reduced in mouse models of liver tumors of various etiologies and in cohorts of human HCC patients. Overexpression of miR-107 or inhibition of its novel target Kinesin family member 23 (Kif23) significantly reduced proliferation by interfering with cytokinesis, thereby controlling survival and motility of mouse and human hepatoma cells. In humans, KIF23 expression was found to be a prognostic marker in liver cancer, with high expression associated with poor prognosis. HDTVi of vectors carrying either pre-miR-107 or anti-Kif23 shRNA inhibited the development of highly aggressive cMyc-NRas-induced liver cancers in mice.

Conclusions Disruption of the miR-107/Kif23 axis inhibited hepatoma cell proliferation in vitro and prevented oncogene-induced liver cancer development in vivo, offering a novel potential avenue for the treatment of HCC in humans.

P4.34 Screening for putative actionable targets reveals changes in PD-L1 expression pattern throughout biliary carcinogenesis

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Intraductal papillary neoplasms (IPN) are a well-defined group of precursor lesions of biliary tract carcinoma (BTC) whose significance in the multistep model of biliary carcinogenesis is still vastly unexplored. Programmed death-ligand 1 (PD-L1) is a primary immune inhibitory molecule expressed on both tumour and immune cells and plays an important role in promoting immune evasion. This study aimed at studying the evolution of PD L1 expression during IPN-related biliary carcinogenesis. METHODS: Intraindividually corresponding high-grade IPN (n = 65), including 54 IPNB, 11 ITPN and their associated invasive BTC (n = 46) were selected for TMA construction. Immunohistochemistry and chromogen-in-situ-hybridization were employed to assess the expression of various targetable biomarkers, including p16, p53, c-myc, c-met, EGFR, HER2, BRAF and PD-L1. These results were correlated with our previously studied spatiotemporal evolution of the immune microenvironment during IPN-associated carcinogenesis focusing on infiltrating immune cell populations. RESULTS: In 6.2 % BTC patients including only distal cholangiocarcinoma, IPN cells expressed PD-L1 at a Tumor Proportion Score (TPS) cut-off of 1 %. Higher PD-L1 levels above 1 % and 10 % Combined Positive Score (CPS) also considering PD-L1 expressing immune cells were reached in 7.7 % and 50.8 % of BTC cases, respectively. At a 1 % cut-off, TPS was associated with distinct histomorphological IPN-subtypes and UICC-Stages. PD-L1 expression in IPN was associated with increased total, stromal and intraepithelial CD3 + -, CD4 + - and CD8 + -immune cell densities. In contrast, only a significant correlation between PD L1 expression and CD8 + -cell density was found in the corresponding BTC tissue. We here showed that PD-L1 expression undergoes dynamic changes throughout IPN-driven carcinogenesis.

P4.35 Proliferative and Metabolic Reprogramming by the Lysine-Specific Demethylase 1 in Hepatocellular Carcinoma

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Lysine-specific demethylase 1 (LSD1) mediates chromatin remodeling by demethylating histone 3 lysine 4 and 9 (H3K4/K9), resulting in gene activation and suppression, respectively. Overexpression of LSD1 in various cancers, including hepatocellular carcinoma (HCC), contributes to cancer progression and malignancy.

In the present study, we show that pharmacological or siRNA-mediated LSD1 inhibition in hepatoma cells leads to altered H3K4/K9 methylation and to cell cycle arrest. Notably, transcriptomics by ultra-deep RNA sequencing and comprehensive proteomics by mass spectrometry, both linked to pathway analysis, revealed a definite impact of LSD1 on cell cycle progression and lipid metabolism. Subsequent chromatin immunoprecipitation (ChIP) followed by whole genome sequencing provided evidence of alterations in the histone methylation signature and LSD1 binding at the promoter sites of genes, involved in proliferation and lipogenesis, that are markedly downregulated after LSD1 inhibition. Noteworthy, ChIP-PCR assays confirmed changes in histone methylation and LSD1 binding at the promoter sites of metabolic genes such as FABP5 and proliferative genes such as PLK1, indicating that they are direct targets of LSD1 regulation. To investigate the effects of LSD1 in vivo, we induced hepatocarcinogenesis based on metabolic liver disease using a DEN/high-fat diet mouse model. Pharmacological inhibition of LSD1 resulted in weight loss, low fat accumulation, and significant reduction in tumor growth and in the number of proliferative Ki67-positive cells.

In conclusion, this study highlights that LSD1 is an important mediator in cell cycle control that affects HCC progression not only through cell cycle arrest but also through metabolic and lipid dysregulation.

P4.36 Anti-Tumor Activity of Cytosolic- and Mitochondrial-Targeted HSP90 Inhibitors in Intrahepatic Cholangiocarcinoma.

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Intrahepatic cholangiocarcinoma (iCCA) is a fatal cancer of the hepatobiliary tract with increasing incidence globally. Therapeutic options are few and frequently ineffective due to tumor intrinsic and acquired chemoresistance. The molecular chaperone heat shock protein 90 (HSP90) plays a prominent role in post-translational maturation and activation of oncogenic clients; especially the mitochondrial pool of HSP90 (mtHSP90) is recognized as critical facilitator of cancer cell growth, survival, and invasive potential. In many cancers, overexpressed HSP90 is a poor prognostic factor. Herein, we investigated the therapeutic potential of HSP90 in iCCA in vitro utilizing the TCGA database, immunohistochemistry, proliferation-, apoptosis-, and cell cycle-assays, mitochondrial respiration tests, and immunoblotting. Immunohistochemically, we discovered elevated Hsp90α/β levels in iCCA precursor lesions, full-blown tumors, and metastases. We chemically targeted HSP90 in various iCCA cell lines using the cytoplasmic inhibitor Ganetespib and the mitochondria-targeting inhibitor Gamitrinib. Gamitrinib demonstrated efficient anti-proliferative and pro-apoptotic activity in all cell lines tested. Oppositely, Ganetespib showed cytotoxicity only in "sensitive" cell lines and limited cytotoxic success in "resistant" cell lines. Combination with mTOR inhibitor MLN0128 and Bcl2 family protein inhibitor Navitoclax ameliorated the cytotoxic effect only of Ganetespib. Gamitrinib had a minor impact on oncogenic signaling pathways, while Ganetespib-sensitive cells, Ganetespib-MLN0128, and Ganetespib-Navitoclax treated "resistant" cells revealed inhibition of mTOR and Bcl-2 anti-apoptotic pathways. Furthermore, Gamitrinib was more potent in disrupting cancer cell mitochondrial activity. Our data highlight that mtHSP90 might be a powerful therapeutic target in iCCA, while combination approaches might be required for effective clinical use of cytosolic HSP90 inhibitors.

P4.37 The effect of methylglyoxal and glyoxalase-1 metabolism on immune-mediated signaling and overall survival in biliary tract cancer

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Introduction Methylglyoxal (MGO) is a cytotoxic metabolite that accumulates in chronic inflammation and cancer, leading to a pro-tumorigenic microenvironment with suppressive effects on immune therapy. Glyoxalase-1 (GLO1) is the rate-limiting enzyme to detoxify MGO and to prevent its accumulation. However, the metabolism of MGO and GLO1 and its effect on overall survival (OS) and immune-mediated signaling in biliary tract cancer (BTC) remains unknown.

Methods Immunohistochemical staining (semiquantitative evaluation) of GLO1 in resected BTCs from 245 patients, and its association with OS was performed. Investigation of MGO and GLO1 was conducted in BTC cell lines (EGI-1, TFK-1, and SNU-1079) using RNA techniques (qPCR) under treatment conditions (GLO1 inhibitor and chemotherapy with gemcitabine/cisplatin). In addition, knockdown of GLO1 (siRNA) and simultaneous treatment with MGO were conducted to analyze the effect on immune-mediated signaling pathways (STAT3, STAT6, and IL-6) and PD-L1 expression.

Results Nuclear staining of GLO1 was associated with better OS in intrahepatic BTC compared to no staining ($p = 0.018$). Inhibition of GLO1 in BTC cell lines resulted in upregulation of IL-6. Treatment with gemcitabine and cisplatin resulted in upregulation of IL-6 and PD-L1. Knockdown of GLO1 led to the upregulation of immune-mediated signaling pathways (STAT3, STAT6 and IL-6). Simultaneous GLO1 knockdown with MGO treatment was associated with a concentration-dependent reduction in PD-L1 (35 % less compared to no treatment).

Conclusions Nuclear GLO1 staining is associated with better survival in intrahepatic BTC. Impaired MGO-GLO1 metabolism may maintain a pro-tumorigenic immune-mediated microenvironment. Understanding these mechanisms may serve to improve immune therapy in BTC.

P4.38 Prognostic and Functional Role of the Glutamine Metabolism in Intrahepatic Cholangiocarcinoma

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Intrahepatic cholangiocarcinoma (iCCA) is the second most common hepatic malignancy. Therapeutic options are limited for this aggressive tumor type due to a lack of druggable drivers, resulting in a poor 5-year survival. One particular feature of cancer cells is the metabolic reprogramming of glutaminolysis, which contributes to energy and macromolecule homeostasis of several tumor types, including iCCA. YAP/TAZ, members of the Hippo pathway ubiquitously activated in iCCA, can coordinate glutaminolysis modulating metabolic enzymes such as glutaminase (GLS1). Herein, we investigated the expression levels of glutaminolysis members SLC1A5, SLC7A5, SLC38A1, and GLS1 in human iCCA specimens dependent on the YAP/TAZ status utilizing Real-time PCR, immunoblotting, immunohistochemistry, and a Glutamine/Glutamate assay. The cytotoxic effect of the selective SLC1A5 competitor V-9302 and the GLS1 inhibitor Telaglenastat, alone or combined, was determined on HuCCT1, KKU213, and RBE iCCA cell lines, and the correlation of glutamine pathway members and YAP/TAZ expression in murine iCCA models overexpressing AKT/YAP or AKT/TAZ. In sum, we found significantly augmented expression levels of SLC1A5, SLC7A5, SLC38A1, and GLS1, as well as YAP/TAZ in human and mouse iCCA specimens. SLC1A5, SLC7A5, YAP, and TAZ expression was inversely correlated with patient's prognosis. Knockdown of YAP/TAZ in iCCA cell lines led to decreased SLC1A5, SLC7A5, and SLC38A1 levels, and diminished glutamate production. Both inhibitors demonstrated anti-proliferative activity, with V-9302 being the most effective, also synergizing with the anti-mitochondrial drug Devimistat in vitro and in ovo. The present data indicate that glutaminolysis is a negative prognostic factor and a potential actionable target in iCCA.

P4.39 Interfering with ß-catenin-induced immunosuppression in HCC by cytokine-armed virotherapy

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Mutations of β-catenin are frequent in HCC and are involved in dysfunctional antigen presentation, immunosuppression, and insensitivity to immunotherapies. Animal models of HCC with defined immunogenicity and dysfunctional Wnt/β-catenin activation are lacking that are suitable for investigations on local immunotherapies including virotherapy. First, we induced HCC In C57BL/6 mice in-situ by hydrodynamic injection (HDI) or local electroporation (EP) of plasmids to deliver SB13-transposase and transposons encoding c-Myc, shRNAp53, mutated p53-R172H, with or without ΔN90-β-catenin. To introduce

immunogenicity, c-myc expression was genetically linked to immunogenic (neo)epitope arrays. After HDI, ΔN90-β-cat expression was essential for develoment of immunogenic HCCs. After EP, HCC occurred in both groups but significantly faster and more reliable in the presence of ΔN90-β-cat. Reduction of EP-induced tissue damage by lowering the voltage almost fully prevented tumor development without mutant β-cat suggesting that the presence of mutated β-cat was decisive in this setting to enable tumor escape from immune surveillance. For a s.c. tumor model, we established the primary cell line HepM-683 from ΔN90-β-cat activated HCC. In this model, we investigated local virotherapy using the oncolytic adenovirus hTert-Ad together with various adenoviral vectors for immunoactivating cytokines. Compared with virotherapy alone, additional expression of Mip1α, Flt3L together with Xcl1, or CCL5 respectively, showed successful TME activation and resulted in reduced growth of β-cat-dysfunctional tumors. Together, we have established syngeneic in-situ and s.c. murine HCC models featuring immunogenic epitopes and β-cat-dependent growth characteristics. These models are suitable for investigations of local immunotherapies such as virotherapy to overcome mut-β-cat-dependent immunosuppression in HCC.

P4.40 Portal and Hepatic Vein Embolization in Perihilar Cholangiocarcinoma – Insights from the DRAGON and EuroLVD Registries

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Background Surgical resection is vital for perihilar cholangiocarcinoma (pCCA). The need for extensive resections often leads to complications, with up to 17 % of patients experiencing post-hepatectomy liver failure (PHLF). To mitigate these risks and enhance Future Liver Remnant (FLR) volume, double vein embolization (DVE), specifically Simultaneous Portal and Hepatic Vein Embolization (PVE/HVE) and Liver Venous Deprivation (LVD), have been introduced.

Methods Data were sourced from the DRAGON Trial Collaborative and EuroLVD registry, evaluating pCCA patients who underwent DVE from 2016-2023. Primary endpoints are resection rate and embolization safety. DVE technique and volumetry were conducted following institutional protocols.

Results Out of 33 patients, 20 (61 %) had Bismuth type III or IV pCCA. After DVE, FLR augmented by 17 % within roughly 16 days. Complications post-DVE encompassed re-drainage (n = 1), cholangitis (n = 3), and two fatalities – one attributed to cholangiosepsis and the other to tumor progression. Following DVE, 67 % underwent resection, with 59 % attaining R0 resections. Major complications were reported in 32 %, yet no deaths occurred within the initial 90 days post-surgery. Median survival post-DVE was 617 days for those operated on, and 148 days for unresected patients.

Conclusion PVE/HVE or LVD in patients with pCCA effectively enhances preoperative FLR volume in a relatively short period and demonstrates low morbidity. In patients undergoing resection, morbidity and mortality rates align with contemporary benchmarks for standard major hepatectomy. The randomized DRAGON 2 PLC trial will assess FLR hypertrophy efficacy, survival, quality of life, and cost-effectiveness of PVE/HVE compared to PVE.

P4.41 Prognostic significance of platelet count in HCC patients adjusted for surrogate parameters of portal hypertension

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Background The treatment of hepatocellular carcinoma (HCC) is often complicated by comorbidities arising from portal hypertension, such as ascites and variceal bleeding. Recent studies have demonstrated a paradoxical relationship between low platelet count, a well-established surrogate marker of portal hypertension, and improved survival in advanced HCC patients. Given this ambivalent association, it is crucial to comprehensively evaluate the role of platelet count in determining patient survival, considering its dual significance as a marker of both impaired liver function and portal hypertension. This assessment should be conducted through a multivariate analysis that encompasses independent markers of portal hypertension.

Aims and Methods To address this, we conducted a retrospective analysis of data from 1,117 consecutive patients diagnosed with HCC between 2006 and 2022, obtained from three medical centers (Munich, Vienna, Mannheim). A multivariate analysis, encompassing various clinical variables including variceal status and spleen size, was performed to assess the prognostic importance of platelet count, independent of its role as a marker of portal hypertension.

Results The multivariate analysis revealed that low platelet counts independently predict survival (OR 1.003; 95 %CI 1.001-1.005; p = 0.014), irrespective of spleen size and variceal status.

Conclusion Our findings validate prior studies suggesting that platelets may exert a detrimental influence on patient outcomes by interacting with the tumor microenvironment, potentially promoting tumor proliferation or metastasis. This data warrants further investigations of the molecular pathways underlying this phenomenon.

Poster Visit Session V VIRAL HEPATITIS AND IMMUNOLOGY 27/01/2024, 11.00am–11.40am

P5.01 Efficacy and Safety at 96 weeks of Bulevirtide 2 mg or 10 mg Monotherapy for Chronic Hepatitis Delta: Results From an Interim Analysis of a Phase 3 Randomized Study

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Federation; 8 Stavropol Regional Hospital; 9 LLC Medical Company "Hepatolog"; 10 Federal State-Funded Institution of Higher Education; 11 Limited Liability Company "Clinic of Modern Medicine"; 12 Gilead Sciences, Inc.; 13 University Medical Center Hamburg-Eppendorf, Medical Clinic Study Outpatient Hepatology; 14 University Hospital Frankfurt; 15 Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Center for Liver Disease, University of Milan

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Bulevirtide (BLV) is a first-in-class inhibitor for chronic hepatitis delta (CHD). Week (W) 48 primary endpoint analysis for MYR301 (NCT03852719), a Phase 3 randomized study, showed monotherapy with subcutaneous BLV at 2 or 10mg/d was superior to no active anti–hepatitis delta virus (HDV) treatment and generally well tolerated. We present findings from the predefined W96 interim analysis.

Patients with CHD were randomized (1:1:1) and stratified based on the presence/absence of compensated cirrhosis as follows: Arm A, no active anti-HDV treatment for 48 weeks followed by BLV 10mg/d for 96 weeks (n = 51); Arms B and C, immediate treatment for 144 weeks with BLV at 2mg/d (n = 49) or 10mg/d (n = 50), respectively, with 96 weeks of follow-up after end of treatment. Combined response was defined as undetectable HDV RNA or decrease by ≥ 2 log10 IU/mL from baseline and alanine aminotransferase (ALT) normalization; other endpoints included viral response, ALT normalization, log10 change in HDV RNA, and change in liver stiffness by transient elastography.

Of 150 patients, 143 (95 %) completed 96 weeks of treatment. W96 efficacy responses were improved vs W48. At W96, similar combined, virologic, and biochemical responses were seen in arms B and C. BLV was well tolerated; there were no drug discontinuations, serious adverse events or deaths attributed to BLV. Increases in bile acids without a correlation to pruritus or other symptoms were noted with BLV treatment. More injection site reactions occurred in patients receiving 10mg/d dosing.

BLV continues to be safe and well tolerated as monotherapy for CHD through W96.

P5.02 Tissue-specific chimeric antigen receptor modified regulatory T cells for the therapy of hepatic inflammation

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Autoimmune hepatitis (AIH) is a chronic inflammatory autoimmune disease of the liver. It involves a T-cell mediated autoimmune response against liver autoantigens, which leads to a loss of tolerance. AIH requires Lifelong immunosuppressive therapy, but this does not lead to a cure for AIH. For this reason, new therapeutic options are needed. One therapeutic approach is cell therapy with regulatory T cells (Treg). The aim of this cell therapy is the restoration of immunotolerance between regulatory and effector T cells. The genetic modification of Tregs with chimeric antigen receptors (CAR) can induce immunological tolerance specifically in inflamed tissue and thus serve as a possible therapy against immune mediated diseases. The CARs contain a tissue-specific single chain fragment (scFv), which ensures enrichment in inflamed liver tissue. To generate liver-specific CAR Tregs, highly specific scFvs were isolated by phage display. These scFvs were tested for specific binding of murine as well as human protein. Different second generation CAR constructs were engineered with these scFvs, which contain two intracellular signaling domains linked to a transmembrane domain. Downstream activation is triggered by specific binding of the scFvs to the target protein. The functionality of the CARs is tested in an NFAT-GFP reporter T cell line. This assay showed that the CARs specifically recognize the antigen without any autoreactivity. As proof of concept studies, the CAR Tregs were tested in disease relevant mouse models of AIH with a focus on stability, migration and effectiveness. In conclusion liver-specific Tregs are a promising therapeutic option for AIH.

P5.03 A tissue-rheostat induced by hepatic cells limits antiviral CD8 T cell immunity during persistent infections

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Persistent HBV infections exhibit limited antiviral CD8 T cell responses characterized by a scarcity of virus-specific CD8 T cells with reduced effector functions. We recently identified a liver tissue-rheostat that curbs virus-specific CD8 T cell function via adenylyl cyclase/cAMP/protein kinase A (PKA) axis. Thus, we aimed to examine the local regulation of the hepatic cell-mediated tissue-rheostat on virus-specific CD8 T cell immunity.

To induce acute-resolving or persistent infection, we used hepatotropic, recombinant adenoviruses to express ovalbumin in hepatocytes. Using transferred, naïve ovalbumin-specific CD45.1 + CD8 T cells, we conducted analysis of liver tissue sections using confocal microscopy. Furthermore, we examined ex vivo or co-culture samples using flow cytometry and tandem mass spectrometry.

Dysfunctional virus-specific CD8 T cells exhibited closer physical interaction with liver sinusoidal endothelial cells (LSECs) and Kupffer Cells (KCs) during persistent infection compared to functional CD8 T cells following resolved infection. Interaction with LSECs and KCs led to upregulation of CXCR6 expression, a tissue-residency marker. Consistently, dysfunctional CD8 T cells during persistent infection displayed higher levels of CXCR6 expression compared to resolved infections. Furthermore, co-cultures with LSECs increased PKA phosphorylation, indicating that cAMP signaling induced by LSECs contributes to local regulation during persistent liver infection. Mass spectrometry further revealed distinct profiles associated with the tissue-rheostat function of LSECs on virus-specific CD8 T cells.

Our findings show crucial spatial relationships between CD8 T cells and hepatic cells during hepatotropic infections. Further research will focus on elucidating the precise molecular mechanism underlying the metabolite-induced tissue-rheostat regulation of T cell function.

P5.04 MK2 and MK3 control anti-viral responses of macrophages to CMV infection while maintaining early viral replication

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The liver is frequently exposed to pathogens via the bloodstream. Therefore, the presence and proper activation of effector cells of the innate immune system such as macrophages is essentially for an initial rapid defense response by releasing cytokines. Many macrophages constitutively reside in the liver, but another large proportion rapidly migrates in from the circulation upon microbial infection. However, macrophages must be tightly regulated since overshooting cytokine production would cause excessive inflammation associated with organ damage. Such complications can occur in some microbial infections. Therefore, we aim to understand the involved signaling pathways to devise therapeutic strategies that limit inflammatory processes without impairing pathogen elimination. Based on a mouse cytomegalovirus (MCMV) infection model, this study focuses the intracellular MAPK-activated protein kinase system (MK)2/3 and its downstream target tristetraproline (TTP). Our data reveal

that disruption of this pathway leads to abrogation of MCMV-induced infiltration of macrophages into the liver and to an interrupted production of many inflammatory cytokines such as TNF-α, IL-6, CCL2 and type I/II interferons (IFNs). Simultaneously, important mediators of NK cell activation and T-cell recruitment such as IL-12 and CXCL9 show enhanced expression. Despite the diminished synthesis of numerous anti-viral effector molecules, this change results in a marked reduction in viral replication! Moreover, macrophages are prevented from adopting their MCMV-induced polarization state under MK2/3-deficient conditions. Taken together, our results suggest that the host MK2/3 system controls a cytokine milieu that is decisive with regard to the immediate and sustained immune control of viruses that infect the liver.

P5.05 Propagation of hepatitis E virus in human neuronal cells as infection model system for extrahepatic manifestations

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Background Historically, Hepatitis E virus (HEV) was described as a hepatotropic virus, but has recently been linked to various extrahepatic manifestations including neurological disorders. Yet, underlying pathogenesis of these neurological injuries remains largely unknown. The aim of this study was to investigate whether neuronal cell lines support the full replication cycle of HEV and to develop a neuronal model system using induced primary neurons (iPNs) to authentically study extrahepatic HEV manifestations.

Methods Five human neuronal-derived cancer cell lines and human iPNs, derived from urinary renal epithelial cells, were used in this study. Employing a state-of-the-art HEV genotype 3 cell culture model, quantification of viral infection of enveloped and naked HEV particles was possible.

Results All neuronal-derived cell lines were susceptible to HEV infection and able to produce infectious particles, demonstrating that HEV can complete its entire replication cycle in neuronal cancer cells in-vitro. Moreover, iPNs and their neuronal progenitor cells supported HEV entry and replication. Volumetric three-dimensional reconstitution showed that mainly differentiated neuronal cells were more susceptible compared to non-differentiated cells. In addition, transcriptomic analysis revealed comparable expression levels of antiviral innate immune signalling genes in infected and uninfected iPNs.

Conclusions In summary, our results indicate that neuronal cell lines are capable of supporting the entire replication cycle of HEV. Moreover, iPNs are susceptible to HEV and serve as a model system as they provide an authentic cellular background for neurological HEV studies and thus the opportunity to gain deeper insights into the relationship between HEV and extrahepatic manifestations.

P5.06 Role of a G-CSF/IL-13 Ratio and Cytokine Dynamics in ALT Flares

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Hepatitis flares can be challenging in the management of these patients, as the clinical outcome of flares (defined as an increase of ALT > 5x ULN) cannot be predicted. To date, there is a lack of knowledge on the immunological pathogenesis and soluble biomarkers for the outcome of hepatitis flares. To investigate this, we performed a Luminex Multiplex assay with plasma drawn from patients with Hepatitis Flares (n = 49 patients).

Comparing the timepoint with ALT > 5x ULN with a follow up timepoint, we see a significant increase of proinflammatory mechanisms as IL-18, CXCL10 (IP-10) or IL1RA in flares. Most significant correlations with biochemical markers of liver injury and dysfunction are seen with IL-18 and G-CSF plasma levels. Interestingly, IL-13 was the only soluble marker correlating negatively with the Hepatocyte Injury Index (HIX). ILC2 cells, considered to be an IL-13 source within the liver, show an impaired phenotype and are significantly decreased in peripheral blood of HBV flare patients.

A ratio of G-CSF to IL-13 parallels with the HIX, ALT levels and the MELD score in these patients. Moreover, by grouping samples and the clinical status of the patients into severity groups, a stepwise significant increase of the G-CSF/IL-13 ratio is seen.

To conclude, we identified the G-CSF/IL-13 ratio as a possible severity marker in patients with flares, indicating a potential role of a complex immune regulation between different immune subsets (e.g. ILCs and neutrophils).

P5.07 Unleashing Synergistic T Cell Power: Novel Insights into Cooperative Killing of Virus-Infected Hepatocytes by Conventional and Auto-Aggressive CD8 T Cells

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Hepatitis B virus (HBV) infections in the liver are typically cleared by the adaptive immune system. However, the limited presence of virus-specific CD8 T cells at the infection site presents challenges. Recently, auto-aggressive T cells, a novel form of metabolic T cell activation, were discovered in nonalcoholic steatohepatitis (NASH). However, their role in viral liver infections remains unknown. Therefore, the aim of this project is to investigate the relevance of auto-aggressive T cells in clearing virus-infected hepatocytes, providing insights into HBV clearance in the absence of high numbers of virus-specific T cells.

To assess the synergistic engagement of antigen-specific CD8 T cells with auto-aggressive CD8 T cells in killing virus-infected hepatocytes, we employed time-lapse cytotoxicity assays measuring cell impedance. Co-cultures of primary murine hepatocytes were established with conventional effector CD8 T cells and auto-aggressive CD8 T cells. By comparing antigen-specific killing with auto-aggressive killing of hepatocytes, we determined their respective contributions.

As expected, antigen-specific CD8 T cells efficiently eliminated virus-infected hepatocytes. Additionally, auto-aggressive T cell mediated killing resulted in antigen-independent hepatocyte elimination at a high effector-to-target ratio (> 1:10). Notably, co-incubation of cytotoxic effector CD8 T cells and auto-aggressive CD8 T cells at ratios, that individually would not induce hepatocyte killing, led to efficient elimination of hepatocytes. Furthermore, we identified soluble factors secreted by conventional T cells that triggered auto-aggression in CD8 T cells. Our findings demonstrate the synergistic effect of cytotoxic CD8 T cells and auto-aggressive CD8 T cells in effectively eliminating virus-infected

P5.08 Liver immunity index: predicting immune control of chronic hepatitis B from circulating CD8 T cells

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Chronic hepatitis B is characterised by a scarcity and dysfunction of virus-specific CD8 T-cells in the liver. Immunotherapies, such as therapeutic vaccination, thus aim to reinvigorate and expand intra-hepatic virus-specific CD8 T-cell immunity to control viral infection. Given the paucity of liver biopsies, we sought to identify markers on circulating virus-specific CD8 T-cells that reflect the immune response at the site of infection and could be used for immune monitoring of immune therapies. Using pre-clinical models of adenoviral hepatocyte-specific delivery of HBV genomes, we studied the dynamics of virus-specific T-cell responses by flow cytometry and single-cell RNA sequencing (scRNAseq).

Following adenoviral transduction of hepatocytes, single-cell transcriptomic and protein-level analysis detected generation of CXCR6 + virus-specific CD8 T-cells in the liver with a tissue-resident phenotype. During an acute-resolving infection, CXCR6 + CD8 T cells were potent effector cells, expressing high levels of Granzyme B and TNF/IFNγ after ex vivo stimulation. CXCR6 + CD8 T-cells in a persistent infection of the liver were rendered dysfunctional. Strikingly, the phenotype and functionality of CXCR6 + CD8 T-cells circulating in peripheral blood were identical to their counterparts in the liver, and predicted infection outcome. Notably, CXCR6 + CD8 T-cells developed solely when recognising their antigen in the liver and not after systemic vaccination in the absence of infection. Differential gene expression analysis between CXCR6 + CD8 T-cells in blood of a resolving vs persistent infection, identifies select parameters which enable prediction of the intra-hepatic immune response during immune monitoring of patients with chronic HBV.

P5.09 HCV rewires NF-kB target gene expression such as CXCL8 involving suppression of endogenous negative regulators of NF-kB-signaling

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The fact that HCV leads to persistent infection in up to 70 % of affected individuals suggest that unique mechanisms have evolved that allow HCV to subvert the host's inflammatory and antiviral response by directly interfering with host cell signal-transduction. This also results in an altered host cell response towards inflammatory mediators such as IL-1β, the serum concentration of which is elevated in patients chronically infected with HCV and correlates positively with viral load. The aim of the study summarized here was to identify possible mechanisms by which HCV mediates increased production of CXCL8 in the host cell in response to IL-1β stimulation.

Based on in silico analysis of the CXCL8 promoter, eight transcription factors whose expression is affected by HCV were identified in addition to NF-kB. Thus, infection with HCV significantly increases the expression of the transcription factors c-Fos, c-Jun, Fra1, IRF1, and ATF3, while significantly decreasing the expression of c-Myb, NKRF, and Oct1. Of these NKRF, Oct1, c-Fos, and c-Jun, together with NF-kB, are involved in the regulation of CXCL8 expression by IL-1β as suggested by knock-down of gene expression using specific siRNA. In this context, it is particularly intriguing that HCV with NKRF and Oct1 represses the expression of factors that are endogenous feedback inhibitors of NF-kB signalling.

In conclusion these data suggest that enhancement of IL-1β-inducible CXCL8 expression by HCV involves increased induction of c-Fos and c-Jun expression as well as enhancement of NF-kB-dependent signaling due to repression of the endogenous feedback inhibitors NKRF and Oct1.

P5.10 Enhancing HBV-Specific T Cell Responses through Epigenetic Modulation and Immune Checkpoint Inhibition in Chronic HBV Infection

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Chronic HBV infection (CHB) leads to exhaustion of HBV-specific T cell responses and epigenetic imprints. These epigenetic signatures prevent a robust immune response. Given the limited success of immune checkpoint inhibitor therapies (e.g., αPD-L1), we aim to target this epigenetic imprinting with the epigenetic modifying DNA methyltransferase inhibitor decitabine (DAC) in combination with αPD-L1 to assess whether the combination can improve HBV-specific T cell immune responses.

We performed 10-day in-vitro culture with peripheral blood mononuclear cells (PBMC) from 56 CHB patients and stimulated the cells with either HBV core overlapping peptide pool (n = 54) or HLA-A2-restricted peptides (n = 22), core18 and pol455. PBMCs were treated with DAC with or without the addition of αPD-L1 on day 3 post-stimulation. The IFNγ response of CD4 + and CD8 + T cells was analyzed by flow cytometry.

DAC and αPD-L1 combination could indeed improve IFNγ responses. The HBV core-specific CD4 + IFNγ response showed an 2- to 100-fold increase by the combination of DAC/αPD-L1 compared with αPD-L1 in some patients, but the responses were highly heterogeneous. Interestingly, DNA methylation analysis of PBMC of the top responders and top nonresponders revealed different epigenetic patterns. For CD8 + T cell responses, pol455-specific CD8 + T cells showed significantly enhanced response to DAC/αPD-L1 compared with αPD-L1 alone (effect size d = 0.47), whereas core18 responses were highly heterogeneous.

In conclusion, our data support the importance of epigenetics in T cell exhaustion and that modification of epigenetics in combination with immune checkpoint inhibitors has the potential to enhance HBV-specific T cell restoration.

P5.11 Antibodies to food antigens contribute to hypergammaglobulinemia in patients with decompensated liver cirrhosis

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Portal hypertension is the major driver in disease progression from the compensated stage to the decompensated stage of liver cirrhosis. Hypergammaglobulinemia (HGG), characterized by elevated immunoglobulin G (IgG) levels, is a common feature of decompensated liver cirrhosis (dCirr). However, the mechanisms underlying HGG and their antigen specificity are incompletely understood. With its immune tolerant environment, the healthy liver mediates local and systemic tolerance to self and foreign antigens, including food antigens. We hypothesize that ingested food antigens bypass the liver in the context of portal hypertension, thereby failing to undergo tolerization and subsequently eliciting immune responses.

We analyzed food-specific IgGs against 90 different food antigens in a cohort of 11 healthy controls (HCs), 22 individuals with dCirr and 8 with a transjugular intrahepatic portosystemic shunt (TIPS). As the generation of IgGs is a T-cell-dependent process, we analyzed T-cell markers of tolerance induction as well as food antigen-specific T-cell responses targeting immuno-dominant regions of four different food antigens in peripheral blood.

Individuals with dCirr showed significantly higher food-specific antibodies (mean values: 9.4 µg/ml in dCirr patients, 16.6 µg/ml with TIPS and 1.9 µg/ml in HCs) with percentages of food-specific IgGs relative to the total IgGs of 1.6 % in HCs, 3.8 % in dCirr and 5.8 % in patients with TIPS. While there was no difference in markers indicative of tolerance induction, food-specific CD4 T-cells were enriched in dCirr compared to HCs.
In conclusion, our data demonstrate that food-specific immune responses might contribute to HGG in dCirr patients with portal hypertension.

P5.12 CXCR6 + PD1 + CD8 T Cells: Key Markers of Liver Inflammation and Dysfunction in ALT Flares

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The contribution of cellular immune responses in the pathogenesis of hepatitis flares (defined as hepatitis with ALT > 5x ULN) has not been fully understood and may be an important target in this setting. Recently, CXCR6 + CD8 + T cells were described as an auto-aggressive T cell subset in mice with NASH (M Dudek et al., Nature 2021). To investigate this concept in patients with hepatitis, we performed spectral flow cytometry analysis with PBMC samples from patients with hepatitis flares (n = 73 patients).
In our cohort, flares were caused by acute, chronic, or non-viral hepatitis. Samples were drawn mostly at two timepoints (admission to hospital and follow-up timepoint). The results were correlated with clinical data and if possible, the Hepatocyte Injury Index (HIX) was calculated for each timepoint.
Hereby, we identify CXCR6 + PD-1 + cytotoxic T lymphocytes as a unique cellular marker in the peripheral blood correlating to liver injury and dysfunction. Comparing CXCR6 + PD-1 + CTLs to total CD8 + T cells, they show are a significantly higher expression of markers associated with activity and potent cytotoxicity. Activity of this subset (marked by HLA-DR expression) correlates with real-time liver injury (HIX), implying a pathogenic role of CXCR6 + PD-1 + CTLs in flares regardless of the etiology. Moreover, plasma levels of CXCL10 correlate with frequency and activity of CXCR6 + PD-1 + CTLs, which could be a potential link.
In conclusion, CXCR6 + PD-1 + cytotoxic T lymphocytes are key players in patients with hepatitis flares and display a potential target for personalized immunotherapeutic approaches.

P5.13 The decrease of HCV-specific neutralizing antibody responses after DAA therapy is associated with weak envelope-specific CD4 T cell immunity

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The need for an effective Hepatitis C Virus (HCV) vaccine remains, despite of the development of direct-acting antivirals (DAAs). Here, we aimed to get a detailed understanding of the longevity and persistence of HCV-specific neutralizing antibodies (nAbs) after DAA-mediated viral clearance and their relation to the CD4 T cell response targeting the HCV-envelope proteins in a longitudinal patient-based study. Thus, we analyzed neutralizing antibodies and T follicular helper (Tfh) cells in a cohort of 27 patients infected with HCV genotype 1a, 1b or 3a.
We found that nAb levels remained stable with some inter-patient variability between baseline and 5-to-7-months post end of therapy and uniformly decreased thereafter. Longitudinal analyses of Tfh cells via flow cytometry revealed stable frequencies before and after DAA therapy. HCV envelope-specific CD4 T cells were determined by secretion of cytokines with flow cytometry after stimulation with in silico predicted genotype-matched envelope peptides. Although, envelope-specific CD4 T cells were detectable in 87.5 % of patients, frequencies of cytokine expressing CD4 T cells were very low. Patients then were ranked based on their baseline nAb response in strong, medium and weak responders. HCV-specific IL-21 producing CD4 T cells, a defining marker for Tfh cells, were only found in chronic HCV patients with strong and medium nAbs. While envelope-specific, IL-21 secreting CD4 T cells were associated with stronger nAb responses in the chronic phase of HCV infection, the overall envelope-specific CD4 T cell response was weak, which might contribute to the failure to maintain nAb levels after DAA-mediated viral clearance.

P5.14 Pegylated interferon reduces relapses following bepirovirsen treatment in participants with chronic hepatitis B virus infection on nucleos(t)ide analogues: end of study results from the Phase 2b B-Together study

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Background In the B-Clear study, bepirovirsen 300 mg for 24 weeks achieved hepatitis B surface antigen (HBsAg) and hepatitis B virus (HBV) DNA < lower limit of quantification (LLOQ) for 24 weeks of bepirovirsen in 9 % of participants on nucleos[t]ide analogues (NA). The B-Together study assessed if sequential bepirovirsen/pegylated interferon (PegIFN) therapy can improve efficacy rates.
Methods Phase 2b, multicentre, randomised, open-label study. Participants on stable NA with HBsAg > 100 IU/mL and HBV DNA < 90 IU/mL were randomised 1:1 to bepirovirsen 300 mg once-weekly (plus loading dose on Days 4 and 11) for 24 (Arm 1) or 12 (Arm 2) weeks, followed by up to 24 weeks of PegIFN 180 mcg once-weekly, with 24 (Arm 1) or 36 (Arm 2) weeks follow-up. Participants continued NA therapy. Primary endpoint: proportion of participants with HBsAg and HBV DNA < LLOQ for 24 weeks after planned end of sequential treatment, in the absence of newly initiated antiviral therapy. Safety was assessed.
Results 108 participants were enrolled (Arm 1 = 55; Arm 2 = 53). Primary endpoint was achieved by 5 (9 %) participants in Arm 1 and 8 (15 %) in Arm 2; all responders had baseline HBsAg ≤ 3000 IU/mL. Only bepirovirsen responders benefited from PegIFN. Bepirovirsen did not appear to adversely influence the safety profile of subsequent PegIFN.
Conclusions Sequential therapy with bepirovirsen and PegIFN improved off-treatment response versus bepirovirsen alone (B-Clear), which may be driven by prevention of relapse in bepirovirsen responders. No new safety signals were reported.
Funding GSK(209348).
Previously presented AASLD2023 (42723).

P5.15 Evidence of durable response to bepirovirsen in B-Clear responders: B-Sure first annual report

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Background In B-Clear (NCT04449029), some participants with chronic hepatitis B virus infection on and not on nucleos(t)ide analogue (NA) therapy (On-NA and Not-on-NA) achieved a response at end of bepirovirsen treatment, sustained for 24 weeks' follow-up. We present preliminary data from B-Sure (NCT04954859) examining durability of response for B-Clear complete responders.

Methods Not-on-NA participants will be followed up at Month 3, Month 6, and every 6 months thereafter (up to 36 months) after B-Clear end-of-study. On-NA participants, if eligible, will cease NA 6 months after B-Clear end-of-study. Adverse events were recorded. Durability of response was assessed:

a) On-NA: Time from NA cessation to loss of complete response.
b) Not-on-NA: Time from achieving a B-Clear complete response to loss of response.

Results 13/16 On-NA and 12/14 Not-on-NA B-Clear complete responders enrolled into B-Sure.

On-NA: 9/13 (69 %) ceased NA—7/9 (78 %) had complete data with ≥ 3 months of follow-up post-NA cessation, and 6/7 (86 %) maintained response; 4/9 (44 %) had complete data with ≥ 6 months of follow-up post-NA cessation, and 100 % (4/4) maintained response. 3/13 (23 %) were not eligible to cease NA, and 1/13 (8 %) withdrew before NA cessation. No participants restarted NAs.

Not-on-NA: 9/12 (75 %) had complete data with ≥ 3 months of follow-up, and 78 % (7/9) maintained response; 3/12 (25 %) had complete data with ≥ 9 months of follow-up, and 100 % (3/3) maintained response.

No safety signals were reported.

Conclusion These data provide early evidence on bepirovirsen durability of response.

Funding GSK (study 206882)

Previously presented at EASL 2023 (abstract 4132).

P5.16 Inflammatory milieu alterations associated with carcinogenesis persist in cirrhotic patients despite HCV cure

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Background Chronic hepatitis C virus (HCV) infection can lead to cirrhosis, hepatocellular carcinoma (HCC) and extrahepatic manifestations. A sustained virological response (SVR) is achieved with direct-acting antivirals (DAA) in over 95 % of the patients, but sequelae of chronic HCV infections do not improve in all patients, suggesting permanent biological alterations. Therefore, we investigated the influence of chronic HCV infection, viral elimination and cirrhosis on soluble immune mediators (SIM).

Methods In 102 chronic HCV patients, 46 with and 56 without cirrhosis, 92 SIM were measured in plasma samples at therapy start, end of treatment and long-term follow-up (median 96 weeks). 8 of the cirrhotic patients developed HCC after the last sampling point. Thirty-nine HBsAg positive persons with HBeAg negative infection served as controls.

Results At baseline, 42 SIM were altered in chronic HCV patients (adj.p < 0.05). Notably, patients with cirrhosis displayed both a higher frequency and more severe alterations. At long-term follow-up, SIM of non-cirrhotic patients were restored, while 41 SIM remained altered in cirrhotic patients (adj.p < 0.05). 33 of these SIM correlated with elastography at follow-up (adj.p < 0.05), among them SIM linked to carcinogenesis as e.g. HGF, IL8 and IL6 (KEGG Pathways hsa05202, hsa05200). Patients developing HCC showed the lowest decline of HGF, uPA, IL8 and IL6.

Conclusions Persisting SIM alterations are closely linked to liver impairment and carcinogenesis. Our results emphasize the need for viral elimination before extended liver damage occurs and suggest the further evaluation of IL6, IL8, CXCL10, uPA and HGF as therapeutic targets.

P5.17 Characterization of HDV-specific antisera with respect to the recognized epitopes

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Introduction Many steps of the Hepatitis D virus (HDV) life cycle remain enigmatic and diagnostic screenings are suboptimal leading to underestimation. Highly sensitive HDV-specific antibodies are needed for research and diagnosis. This study aims to characterize epitopes recognized by HDV specific antibodies.

Methods Two rabbits were immunized with the purified S-HDAg either in its native or in a denatured form. Epitope mapping of obtained hyperimmune sera was performed using peptide arrays synthetic peptides covering the full-length S-HDAg protein.

Results Epitope mapping revealed several highly reactive B-cell epitopes mainly in the C-terminal S-HDAg-region. Antisera obtained by immunization with denatured S-HDAg were more polyclonal as reflected by more recognized epitopes. Projection of these epitopes into a 3D model of S-HDAg revealed shielding of these epitopes in the native state. For instance, epitope DENPWLGNI is localized in the dimerization domain of S HDAg and is shielded in the oligomeric state of native S-HDAg. This prevents recognition in case of immunization with the native protein. In contrast to this, epitope EDERRERRVA is localized within the more accessible RNA binding domain of S-HDAg and therefore is recognized during immunization by native S-HDAg.

Conclusion We identified highly immunogenic S-HDAg epitopes, which are accessible in the native conformation of S-HDAg and can be used for generation of highly specific S-HDAg-specific monoclonals. Furthermore, synthetic peptides bearing these epitopes can be used as potential vaccine candidates. The sera obtained in this study can be used for CLSM-based analysis of HDV life cycle and for quantitative analysis of HDAg.

P5.18 NK T cell-mediated mild hepatitis occurs in Tg1.4HBV-s-rec mice, a crossbred hepatitis B virus-transgenic model

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Hepatitis B virus (HBV)-transgenic mice exhibit competent innate immunity and are therefore an ideal model for considering intrinsic or cell-based mechanisms in HBV pathophysiology.

A highly replicative model that has been little used, let alone characterized, is the Tg1.4HBV-s-rec strain derived from cross breeding of HBV-transgenic mouse models that either accumulate (Alb/HBs, Tg[Alb1-HBV]Bri44) or lack (Tg1.4HBV-s-mut) the hepatitis B surface antigen (HBsAg). Hepatocyte morphology was analysed using transmission electron microscopy (TEM) and con-

focal microscopy. HBV replication was characterised on RNA and protein level. Hepatic immune cells were quantified an characterized.
Tg1.4HBV-s-rec hepatocytes secreted HBsAg, Hepatitis B extracellular antigen (HBeAg) and produced HBV virions. Transmission electron microscopy visualised viral particles (Tg1.4HBV-s-rec), nuclear capsid formations (Tg1.4HBV-s-mut and Tg1.4HBV-s-rec) and endoplasmic reticulum malformations (Alb/HBs). Viral replication in Tg1.4HBV-s-rec and Tg1.4HBV-s-mut differed in HBsAg expression and interestingly in the distribution of HBV core antigen (HBcAg) and HBV x protein. While in Tg1.4HBV-s-mut hepatocytes, the HBcAg was located in the cytoplasm, in Tg1.4HBV-s-rec hepatocytes, the HBcAg appeared in the nuclei, suggesting a more productive replication. Finally, Tg1.4HBV-s-rec mice showed symptoms of mild hepatitis, with reduced liver function and elevated serum transaminases, which appeared to be related to natural killer T cell activation.
In conclusion, the study of Alb/HBs, Tg1.4HBV-s-mut and their F1 progeny provides a powerful tool to elucidate HBV pathophysiology, especially in the early HBeAg-positive phases of chronic infection and chronic hepatitis.

P5.19 The Yin and Yang of γδ T cells in hepatitis B virus (HBV) infection

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Immune responses are critical for controlling viral infections such as hepatitis B virus (HBV) infection, but they are also a mediator of inflammation and disease progression. Human γδ T cells are known to play a role in various infectious diseases and are enriched in the liver, which may suggest a role in liver-associated diseases. However, their role in the context of HBV infection have remained largely unexplored. Therefore, we performed in-depth phenotyping by full-spectrum flow cytometry of γδ T cells in PBMCs of patients with acute and chronic HBV infection (including patients discontinuing therapy) with different viral and clinical characteristics.
We have identified two distinct γδ T cell subsets. CXCR6 + γδ T cells, which are enriched in the liver, show a pro-inflammatory phenotype, including Granzyme K expression, and displayed a positive correlation with markers of liver inflammation in acute HBV patients. CXCR6 + γδ T cells increased in frequency during ALT flares after stopping NUC therapy.
CD16 + γδ T cells demonstrated a negative correlation with Hepatitis B core related antigen level. Intriguingly, these CD16 + γδ T cells expressed cytotoxic effector molecules like Granzyme B, albeit without a corresponding correlation with markers of liver inflammation in acute HBV infection.
Our findings imply dual roles for γδ T cells in HBV infection dynamics. CXCR6 + γδ T cells may contribute to disease pathogenesis, while CD16 + γδ T cells may play a role in facilitating viral control. This intricate interplay underscores the delicate balance between immune responses and their potential impact on HBV infection outcomes.

P5.20 Higher prevalences and titers of CMV, EBV and HSV1 viremia in patients with acute-on-chronic liver failure derived from the ACLF-I cohort

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DOI 10.1055/s-0043-1777645
Background Acute-on-chronic liver failure (ACLF) is a life-threatening clinical syndrome characterized by the rapid deterioration of liver function in individuals with pre-existing chronic liver disease. While various factors contribute to ACLF, emerging evidence suggests that viral infections can serve as potent triggers. Herpesviruses, including CMV, EBV and HSV are highly prevalent viruses. Key factors linking the viruses to ACLF include their abilities to induce cytokine storms, activate immune cells and trigger a pro-inflammatory state.
Methods We analysed blood samples from 212 patients registered in the ACLF-I-study for viremia of the herpesviruses CMV, EBV and HSV by multiplex-PCR. Patients' charts were reviewed for additional clinical data.
Results Higher prevalences of CMV (45 % vs. 30 %), EBV (10 % vs. 6 %) and HSV1 (8 % vs. 1 %) as well as higher viral loads of > 1000 copies/ml regarding CMV (8 % vs. 3 %), EBV (2 % vs. 0 %) and HSV1 infections (6 % vs. 1 %) were observed in ACLF patients in comparison to non-ACLF patients. Viremia remained undetected and there was no clinical suspicion for viral infections documented in patients' charts. In the majority of ACLF patients with detected viremia the precipitant of ACLF was unknown. No significant immunosuppression was documented and no correlation to leucocyte levels could be identified.
Conclusion The observed high prevalence of herpesviruses in our study may reflect a state of immunosuppression in patients with acute-on-chronic liver failure. However, the presence of viral infections may also tip the balance towards ACLF by exacerbating liver inflammation and impairing hepatocellular function in individuals with chronic liver disease.

P5.21 HCV promotes upregulation of NRG1 and EGF gene expression by enhancing Sp1 DNA binding

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Regardless of available treatment, HCV remains a significant risk factor for chronic liver diseases and development of hepatocellular carcinoma (HCC). We have previously demonstrated that HCV induces expression of ligands of the ErbB receptor family, such as Nrg1 and EGF, and interferes with their signal transduction, thereby affecting intercellular communication of the host cell. The molecular mechanisms by which HCV induces the expression of these growth factors have yet to be elucidated.
Based on in silico analyses of the 5' regions of the nrg1 and the egf gene, chromatin immunoprecipitation (ChIP) was used to analyze the impact of HCV on DNA binding of transcription factors potentially involved in the regulation of these factors, such as Sp1, in Huh7 cells for control and in cells harboring the HCV subgenomic replicon (Huh9-13). In addition, the involvement of PI3K- and p38MAPK-dependent signaling in the regulation of nrg1 and egf expression was analyzed using inhibitors.
The data suggest that HCV induces Sp1 binding to identified sites in the promoter region of the nrg1 and egf genes. The regulatory relevance of this observation is supported by the observation that knock-down of SP1 gene expression via specific siRNA leads to reduced expression of nrg1 and egf. In addition, inhibitor studies suggest that the PI3K pathway plays a role in the influence of HCV on the expression of nrg1 while the p38MAPK pathway seems to be relevant for the induction of egf expression.
In conclusion, HCV utilizes host transcription factors like Sp1 to reprogram hepatocyte's cellular response.

P5.22 Hepatitis D Virus Coinfection in Patients with Acute-on-Chronic Liver Failure: Impact on Disease Severity and Survival

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DOI 10.1055/s-0043-1777647

Background Acute-on-chronic liver failure (ACLF) poses a significant clinical challenge, and its etiology, including the role of hepatitis B (HBV) and D virus (HDV) coinfection, remains an area of active research.

Methods We conducted a monocentric, retrospective data analysis of patient records, focusing on virological data, disease characteristics, and sociodemographic factors. The cohort included 570 patients admitted to the intensive care unit (ICU) with ACLF from 2015 to 2022. Statistical analyses, including Kaplan-Meier survival curves and a log-rank test, were performed to assess differences in survival among these groups.

Results In our cohort of ACLF patients a subset (n = 10) had active HDV coinfection alongside patients with active HBV monoinfection (n = 15), HBsAg loss (n = 37) and Anti-HBc only (n = 8), representing 40 % of the patients with replicative HBV infection (n = 25). These patients were younger (mean age 55.7 years) and less severe liver disease or ACLF severity as indicated by lower MELD and CLIF-C ACLF scores (mean MELD-Score 16, mean CLIF-C ACLF-Score 38). Kaplan-Meier survival analysis showed a reduced survival time in trend (12 days) between groups (HBsAg loss: 131 days, Anti-HBc only: 58 days, HBV monoinfection: 19 days, Overall mortality: 83 days).

Conclusion Our findings highlight the underrecognized impact of HDV coinfection in patients with ACLF. Despite their comparatively lower severity of underlying liver disease, patients with HDV coinfection face worse survival outcomes. Hence, further research is needed to explore the mechanisms and distinct overrepresentation of HDV coinfections compared to HBV-monoinfected patients.

P5.23 Evolution of resistance-associated variants in HEV-patients with sofosbuvir mono- or sofosbuvir-ribavirin combinatory therapy

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Background Hepatitis E virus is a single-stranded RNA virus that causes over 20 million infections worldwide. The lack of an HEV-specific antiviral, leaves drug repurposing as a fast-tracked option to help patients with symptomatic infection. Besides off-label use of ribavirin and interferon, sofosbuvir is used to treat chronically infected patients. However, for sofosbuvir and ribavirin treatment relapse often occurs which are associated with the emergence of resistance-associated variants.

Methods We here analyzed HEV population dynamics during sofosbuvir monotherapy or sofosbuvir-ribavirin concomitant therapy in patients using high-throughput sequencing. Dominant variants were identified and subsequently characterized for in vitro replication capacity and drug sensitivity using an HEV replicon system.

Results Our results suggest that viral heterogeneity plays an important role in treatment resistance. In particular, a mutation, A1343V, in the polymerase finger domain that occurred in the majority of patients under sofosbuvir monotherapy resulted in a 5-fold decrease in susceptibility while maintaining replication capacity in vitro. Furthermore, sensitivity to ribavirin treatment remained unchanged. Followingly, we identified similar variants, including the A1343V, in patients treated with a sofosbuvir-ribavirin combination. Despite occurring, this variant was highly sensitive to sofosbuvir-ribavirin combination treatment in vitro.

Conclusions In summary, we identified a sofosbuvir-specific variant in the polymerase that was associated with treatment resistance in infected patients. The variant A1343V was less susceptible to sofosbuvir in vitro without change in replication. Importantly, the resistant phenotype could be overcome in tissue culture by sofosbuvir-ribavirin co-treatment. Therefore, future antivirals should consider viral populations dynamics and variants associated with drug resistance.

P5.24 Metabolic Phenotype and Morbidity in Patients with Primary Biliary Cholangitis

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Background and Aims Primary biliary cholangitis (PBC) is a chronic, autoimmune liver disease that often leads to fibrosis and cirrhosis of the liver. In this population-based cohort study, we aimed to investigate morbidity and mortality together with metabolomic changes of PBC in the UK.

Methods 415 patients with PBC and 830 matched (age, sex, BMI, ethnicity) controls, without liver disease, were included in this study. A subset of patients with PBC and controls were analyzed for their metabolomic profile. Further, we used a Phenome wide association study (PheWas) to elucidate the associated comorbidities. To incorporate for the changes resulting from chronic liver disease and advancement of PBC, we compared the matching with healthy controls to a second matching also including the APRI-score (AST/thrombocyte ratio index).

Results Compared to the control group, various pathways relating the metabolic profile of amino acids, lipids, and liver biochemistry were significantly enriched in patients with PBC. Notably, patients with PBC showed a higher prevalence of cardiovascular diseases, digestive diseases, autoimmune diseases, anemias, mental disorders, and urinary tract infections. Incorporating the APRI-score, indicated that the observed morbidity was not solely attributable to advanced disease state or progression of liver disease.

Conclusions Our study provides a detailed insight into the morbidity of patients with PBC compared to controls on a populational level. The exploration of potential effects of disease state on morbidity suggests that early detection and early treatment of PBC could prevent the onset of comorbid diseases and thereby improve patient prognosis.

P5.25 Role of Vitamin D in Immune Response in Patients with Viral Hepatitis

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Background To study the relationship of Vitamin D with innate and adaptive immune response parameters in chronic hepatitis B and C patients.

Methods The laboratory data between 01.01.2013-01.01.2023 for patients with chronic hepatitis B (CHB), chronic hepatitis C (CHC) were extracted. Serum 25-hydroxyl vitamin D, hepatitis B virus serological markers, complemets and subsets of T lymphocytes were determined.

Results In CHB and CHC patients the percentage of CD4 + T lymphocytes and the CD4 + /CD8 + ratio significantly decreased ($p < 0.05$), but the percentage of CD8 + increased ($p < 0.05$) compared to control group. In CHB patients Vitamin D decrease was significant ($p < 0.001$) but not in CHC patients. The vitamin D

deficient group showed significantly lower antibody production compared to the normal group, and exhibited significantly decreased CD4 numbers and increased CD8 numbers ($p < 0.05$ and $p < 0.001$, respectively). Complement C3 levels had an inverse relation with Vitamin D. Vitamin D levels were significantly associated with complement C3, CD8 + , CD4 + , CD19 + cells, and HBV DNA levels.

Conclusions Vitamin D may be a modulator of immune function not only via CD8 + and CD4 + cells but also via CD19 + cells in the course of chronic HBV infection. Moreover, the increased proportion of B cells and decreased CD4 + cells in Vitamin D deficiency disrupts the immune response against HBV since the expected antibody response was not obtained despite the increase in B cell ratio. This indicates an influence of CD4 + cells for B cell functionality.

P5.26 Unique features of a cytotoxic, protective CD8 + T-cell population in chronic HBV infection

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The phenotypic and functional heterogeneity of HBV-specific CD8 + T cells depends on the targeted antigen and comprises distinct HBV-specific CD8 + T-cell subsets with hallmarks of exhausted CD8 + T cells. However, whether there is a link between the antigen-dependent HBV-specific CD8 + T-cell heterogeneity and the clinical phase of chronic HBV infection, and whether other mechanisms in addition to T-cell exhaustion play a role in HBV-specific CD8 + T-cell dysfunction have not been investigated yet.

Cluster analysis of the single-cell transcriptomes of HBVcore18- and HBVpol455-specific CD8 + T cells revealed a different subset diversification between individuals with HBeAg- chronic HBV infection (n = 7) and NUC-treated HBeAg- chronic hepatitis B (n = 3). Whereas HBV-specific CD8 + T cells of NUC-treated individuals exhibited memory/memory-like T-cell characteristics, we uncovered a novel subset with a cytotoxic signature among HBVpol455-specific CD8 + T cells within the HBeAg- chronic HBV infection phase. Interestingly, these cytotoxic effector-like HBV-specific CD8 + T cells differ from bona-fide HBV-specific effector CD8 + T cells of a self-limiting HBV infection, despite displaying a comparable cytotoxic and functional capacity. Indeed, they exhibit differences in transcriptional regulator expression of the effector T-cell differentiation program, which might be an effect of TGFβ signaling. By blocking TGF-β signaling, the attenuated effector differentiation can be redirected towards a bona fide effector cell program.

In sum, this study sheds light on a functionally adapted CD8 + T-cell population with a potent cytotoxic capacity that is associated with endogenous viral control. Stimulating these cells to exert their full effector function might be a potential immunotherapeutic approach in HBV cure.

P5.27 Cytokine response of natural killer cells to hepatitis B virus infection depends on monocyte co-stimulation

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Objective The hepatitis B virus (HBV) remains a major burden on health systems worldwide. The course of an HBV infection depends on a complex interplay of different immune cell populations. Especially natural killer (NK) cells are an important component of the innate immune response and provide an essential first line of defense against viral pathogens through the production of antiviral cytokines and direct cytotoxicity. Here, we investigated the antiviral immune response of NK cells to in vitro HBV infection and identified monocyte co-stimulation as an essential component.

Design The human hepatoma cell line HepG2 expressing the NTCP receptor was inoculated with HBV followed by co-culture of healthy donor lymphocytes. Viral replication was assessed by PCR and ELISA and lymphocytes were analysed by flow cytometry.

Results Initial studies showed a clear advantage of HBV replication by HepG2-NTCP over other hepatoma cell lines. The co-culture of lymphocytes demonstrated a significantly increased production of antiviral cytokines, with CD56 + + CD16- NK cells in particular producing high levels of IFN-κ. Isolation of NK cells subsequently abolished the effect, which was also evident when lymphocytes were separated from their target cells using transwells. Depletion of CD14 + monocytes also showed an attenuation of the effect. However, a combined culture of isolated monocytes and NK cells restored the cytokine production, while demonstrating the necessity of direct cell contact between these two populations as well.

Conclusion In vitro HBV infection leads to increased cytokine production by predominantly CD56 + + CD16- NK cells, which follows a primarly contact-dependent stimulation of monocytes.

P5.28 Role of Vitamin D in Immune Response in Patients with Viral Hepatitis

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Background To study the relationship of Vitamin D with innate and adaptive immune response parameters in chronic hepatitis B and C patients.

Methods The laboratory data between 01.01.2013-01.01.2023 for patients with cHBV, cHCVre extracted. Serum 25-hydroxyl vitamin D, HBV serological markers, complemets and subsets of T lymphocytes were determined. Study cohort were divided into three groups based on serum 25-hydroxyl vitamin D level with further evaluation of laboratory data.

Results In cHBV and cHCV the percentage of CD4 + T lymphocytes and CD4 + / CD8 + ratio significantly decreased ($p < 0.05$). In cHBV Vitamin D decrease was significant ($p < 0.001$). Vitamin D showed a moderate negative influence on the CD8 cell count in cHBV patients. The positive ratio of HBV-DNA and HBsAg decreased with increasing serum vitamin D levels. The vitamin D deficient group showed significantly lower antibody production compared to the normal group, and exhibited significantly decreased CD4 numbers and increased CD8 numbers ($p < 0.05$ and $p < 0.001$, respectively), while the CD4/CD8 ratio was also significantly decreased in the insufficiency group ($p < 0.001$). Vitamin D levels were significantly associated with complement C3, CD8 + , CD4 + , CD19 + cells, and HBV DNA levels.

Conclusions Vitamin D may be a modulator of immune function via CD8 + and CD4 + cells and via CD19 + cells in chronic HBV infection. Moreover, the increased proportion of B cells and decreased CD4 + cells in Vitamin D deficiency disrupts the immune response against HBV. This indicates an influence of CD4 + cells for B cell functionality.

P5.29 Generation of HBV DNA Plasmids of Genotype E for In Vitro Infection Model

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Background The African specific HBV genotype E (HBVgtE) is associated to a relative high viral replication and greater risk of hepatocellular carcinoma. However, studies concerning this genotype are still under-represented. This project aims at generating replication competent HBV DNA plasmid of genotype E to better understand biomarker production and pathogenesis by this genotype in vitro.

Method The fast cloning was realised following the well-established protocol from Li et al. in 2011. In brief, the primers for insert amplification were designed with insert-specific sequences and additional 15-17 bases overlapping with the vector-ends. The 1.5mer was generated from the 1.1mer template and for the 1.1merG1896A, the mutation G1896A was directly inserted in the primer sequences for 1.1mer vector amplification resulting in the knockdown of HBeAg expression. Then, the recombinant 1.1 and 1.5merHBV-E DNA plasmids were transfected into HepG2-hNTCPcells and their HBsAg/HBeAg Level were measured in the supernatant.

Results Transfection of HBV-E Plasmids resulted in a successful production of HBeAg and HBsAg with a higher expression of both biomarkers in 1.5mer. We found similar meanlevels of HBV DNA, RNA and total HBsAg after transfecting the 1.5mer during 8days at ratio 2:1; 3:1; 4:1. Interestingly, mean LHBs were slightly higher using a transfection ratio 3:1 (2.7ng/mL) compared to 2:1 (0.79ng/mL) and 4:1 (0.72ng/mL) suggesting a better viral fitness of HBVgtE.

Conclusion The newly engineered HBV-Eplasmids can be transfected in cell cultures hereby showing stable levels of biomarkers. Our model is ready-for-use in further in vitro studies on HBV replication and drug efficacy of HBVgtE.

P5.30 Limited stability of Hepatitis B virus RNA in plasma and serum

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Background & Aims Pregenomic hepatitis B virus (HBV) RNA (pgRNA) is intensively investigated as a biomarker in the management of HBV infected patients. However, prior to the use in routine clinical practice potential confounders of test results need to be identified. This study investigates the stability of HBV pgRNA under various storage conditions.

Methods HBV RNA level of 26 hepatitis B virus infected patients, were determined using the Roche cobas® 6800/8800 investigational HBV RNA assay. Supernatant (S) and whole blood (WB) of plasma and serum samples were stored for 6, 48, 169 hours (h) at 4, 25 and 42 °C, respectively. Additionally, 10 serum and plasma samples underwent 4 or 11 cycles of freezing (-80 °C) and thawing (25 °C).

Results A significant decline in mean HBV RNA concentration compared to baseline was observed in samples stored at 25 °C for more than 48h. Under storage at 42 °C, a significant decline in mean HBV RNA occurred already after 6 h of storage. Additionally, sub-analyses of predefined HBV RNA baseline concentrations (≤ 10 cp/mL, > 10-100 cp/ml, > 100 cp/mL) revealed significant changes in the number of samples with detectable HBV RNA as well as in median HBV RNA level after storage at temperatures of 25 and 42 °C. No effect of freezing and thawing on HBV RNA level was observed.

Conclusions A qualitative detection of HBV RNA is feasible in samples with > 100 cp/mL up to 48 h under storage temperatures of 4–42 °C. For most stable quantitative HBV RNA values storage at 4 °C should be preferred.

P5.31 Regulation of SARS-CoV-2 infection by microRNAs in primary human hepatocytes

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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.

P5.32 Unique phenotype of CD8 + T Cell Responses in Acute Hepatitis C Virus Infections

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Hepatitis C virus (HCV) infections can manifest as acute-spontaneously resolving or chronic, with CD8 + T cells (CTLs) playing a pivotal role in disease outcome. While extensive research focused on exhaustion of virus-specific CTLs in chronic HCV infection, the characterization of protective CTL responses has been understudied in recent years. Moreover, past investigations primarily focused on the prevalent European HLA allele HLA-A*02:01 when exploring distinctive phenotypes of HCV-specific CTLs in chronic versus spontaneously resolving infections, yet its clinical association with disease outcome remains weak.

Here, we examined HCV-specific CTLs targeting the HLA-B*27:05-restricted epitope NS5B2841-2849, known for its clinical association with spontaneously resolving disease course. Using magnetic bead-based enrichment and flow

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cytometric analysis, we comparatively profiled HLA-A*02:01 and HLA-B*27:05-restricted HCV-specific CTLs in spontaneously resolved (n = 11 and 9, respectively) and chronic (n = 10 and 8, respectively) HCV patients.
While both spontaneously resolved patient cohorts exhibited a consolidated memory pool of CD127-expressing CTLs, CTLs specific to the HLA-B*27:05 NS5B2841-2849 epitope displayed twofold higher PD-1 expression. This difference was also observed when comparing HLA-B*27:05-restricted CTLs from patients with spontaneously resolved versus chronic-cured infections, further underscoring the unique phenotype of HLA-B*27:05-specific CTLs in the context of self-limiting HCV infections. Additionally, HLA-B*27:05-specific CTLs obtained from chronic-cured patients showed marked reduction in their exhaustion signature, compared to their chronic-cured, HLA-A*02:01-restricted counterparts.
Understanding the unique phenotype of protective CTL responses in spontaneously resolving HCV infections might help to generate important immune correlates of protection and advance our understanding of protective HCV immunity and strategies for vaccine development in the future.

P5.33 Neutrophil-Derived miRNA-223 Modulates Peritoneal Immunity in Patients with Cirrhosis During Spontaneous Bacterial Peritonitis

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DOI 10.1055/s-0043-1777658
Background MicroRNAs (miRNAs) play a pivotal role in modulating inflammatory responses and cell-cell interactions during bacterial infections. This study investigates the alterations in miRNA composition in patients with spontaneous bacterial peritonitis (SBP), identifies cellular sources, and assesses the functional consequences.
Methods Paired serum and ascitic fluid samples from 11 patients, obtained before and during SBP, were subjected to multiplex microRNA profiling. Regulated miRNAs were further validated using LNA miRNA PCR assays. The source of miRNA was assessed by measuring intracellular and supernatant concentrations using cultured neutrophils and mononuclear cells. Functional consequences of miRNA-223 were evaluated using co-culture assays and transfection of THP-1 cells.
Results Multiplex microRNA profiling identified five miRNAs that were differentially regulated in ascitic fluid during SBP. qPCR validation confirmed the enrichment of miR-223 during SBP. While TLR4 ligation down-regulated miR-223 in peritoneal macrophages and did not affect the release into the supernatant, neutrophils could be identified as a potent source of extracellular miR-223. Incubation with neutrophil supernatant affected the canonical inflammasome and NF-κB signaling in human peritoneal macrophages, which is currently being validated in miRNA-223-transfected THP-1.
Conclusion During SBP, miR-223 is released from infiltrating neutrophils and is enriched in ascitic fluid. Peritoneal macrophages can take up neutrophil-derived miRNA, which affects peritoneal inflammation, limits tissue damage, and shapes subsequent immune responses in the peritoneal cavity.

P5.34 Intracellular “in silico microscopes” – fully 3D spatial Hepatitis C virus replication model simulations

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DOI 10.1055/s-0043-1777659
To face present and upcoming virus pandemics and endemics such as the Hepatitis C virus (HCV) induced liver damages, detailed quantitative biophysical understanding of intracellular virus replication mechanisms is necessary. Understanding the interplay of form and function might allow to figure out attack points for the strategic development of direct antiviral agents (DAA) and vaccines. Biophysical research of spatio-temporal evolution of virus replication so far is rare. We are developing a framework to allow for spatio-temporal resolved virus replication simulations based on advanced numerical mathematical methods. This study presents an advanced highly nonlinear model of the HCV genome replication cycle. The diffusion-reaction model describes the interplay of the major components of the viral RNA (vRNA) cycle, which are the non structural viral proteins (NSP), the vRNA, and a host factor. Technically, we couple surface PDEs (sufPDEs) on the 2D Endoplasmatic Reticulum (ER) with PDEs in the 3D cytosol. The model is evaluated at realistic reconstructed cell geometries, the geometries are based on experimental data. The simulations reproduce the effects of NSPs which are restricted to the ER surface with those appearing in the cytosol volume. The visualization of the simulation resembles a look into some sort of "in silico" microscope. The output data approach quantitative experimental data and request experimental model validation. [2]
Literature
[1] dois. doi:10.3390/ijerph16030513, 10.3390/v10010028, 10.3390/v9100282, 10.1007/978-3-031-40864-9 26.

P5.35 ‘Accidental functional cure’: HBsAg loss in a HBV/HDV/HIV-coinfected patient upon non-adherence to treatment with nucleos(t)ide-analogues

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Introduction Discontinuation of nucleos(t)ide analogues in patients with chronic hepatitis B infection is supported by data from the STOP-NUC and RETRACT-B trials, but there is little data on HDV and/or HIV coinfected patients. We present a patient with HBV/HDV/HIV coinfection who experienced a severe relapse after discontinuation of antiviral treatment, resulting in HBsAg loss.
Case Report A 58-year-old male patient with chronic HBV/HDV/HIV-coinfection who was treated with doravirine/lamivudine/tenofovir in our outpatient clinic had an undetectable HIV/HBV viral load, good immunological status (CD4 728/µl), no evidence of cirrhosis (elastography 5,4kPa), low HDV viremia (240IU/ml) and low detectable HBsAg (5IU/ml). In March 2023, he presented to the emergency department with nausea and vomiting, which he had been experiencing for two months. In the hope that his symptoms would improve, he stopped taking his medication. Upon admission, ALT was 5379 U/l and total bilirubin 2.3 mg/dl, HBV-DNA 30,700,000 IU/ml, HIV-RNA 73,000 copies/ml and HDV-RNA had increased to 796,000,000 IU/ml. Endoscopy revealed gastritis, no other cause for his gastrointestinal symptoms was identified. ART (with bictegravir/emtricitabine/tenofovir-alafenamide) was resumed, and antiviral treatment of HDV- infection with bulevirtide was initiated. Monthly checkups showed a steady decrease of HDV-RNA and HBsAg level, resulting in HBsAg loss in September 2023. We plan to continue bulevirtide for three months after HDV-RNA clearance.
Discussion NUC-stopping strategies may be a feasible option in HBV/HDV coinfected patients with low HBsAg levels and no evidence of cirrhosis, provided that close monitoring is performed. In HIV-positive patients, this would require switching to NUC-free ART-regimens.

P5.36 Pegylated interferon-alpha treatment in vivo potently reduces all HDV markers in primary human hepatocytes undergoing cell division

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Background and aims Studies in humanized mice demonstrated that pegylated interferon-alpha (peglFNα) suppressed patient-derived HDV strains (Giersch, JHEPRep 2023). In vitro studies showed that IFNα exerts stronger anti-HDV activities in hepatoma cells undergoing cell division (Zhang, JHepatol 2022). This study aimed to investigate the impact of cell division and peglFNα treatment on HDV replication in vivo.

Methods uPA/SCID/IL2Ry/- (USG) mice received primary human hepatocytes (PHH) isolated from a mouse previously reconstituted with PHH and stably infected with HBV and HDV. From week 1 to 8 after PHH transplantation, half of the mice received peglFNα s.c. (25ng/g biweekly). Cell proliferation, virological markers, and IFN response were analyzed using qRT-PCR, ELISA, and immunofluorescence.

Results In an environment supporting liver regeneration, transplanted PHH underwent strong expansion both in untreated and peglFNα-treated animals. In untreated mice, the overall intrahepatic HDV RNA level increased as PHH expanded, while HDV RNA levels per PHH remained unchanged. In line with previous studies (Giersch, Gut 2019), intrahepatic HBV markers decreased during the first 5 weeks of cell division, resulting in an increased proportion of PHH appearing HDAg-positive but HBcAg-negative. PeglFNα treatment promoted further reduction of intrahepatic HBV DNA and RNA (median 2.7log10 and 2.2log10) and resulted in undetectable levels of HDV viremia, HDV RNA, and HDAg-positive PHH in the liver.

Conclusion Although HDV can spread among PHH undergoing cell division, the study shows that peglFNα does not hinder PHH proliferation in vivo and that the treatment potently reduces HDV.

P5.37 HLA class I-associated mutations are predominantly located in the HBV core protein and restricted to HBeAg-negative HBV infection

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Although selection of escape mutations in CD8 T cell epitopes has been previously described in HBV infection, the overall impact of CD8 T cell selection pressure and its influence on HBV sequence diversity remains unclear. Here, we applied whole-genome sequencing to HBV isolates from 532 HLA class I genotyped patients and detected HLA-associated mutations in viral genomes (HAMs) using a Bayesian model (HAMdetector).

We found strong evidence for HLA class I-associated selection pressure on HBV. Using previously published thresholds for the identification of HAMs, a total of 308 residues showed evidence of CD8 T cell escape, the majority of which were located in previously unidentified epitopes. Interestingly, HAMs were highly enriched in the HBV core protein compared to all other HBV proteins. Consistent with a greater degree of selective pressure on the core protein, HAM scores were also higher in previously described epitopes in core compared to described epitopes in other proteins. Interestingly, the degree of adaptation to HLA class I immune pressure of individual HBV isolates, as determined by a novel "adaptation score", negatively correlated with HBV viral load. Furthermore, the adaptation score was lower in isolates from HBeAg-positive infections compared to HBeAg-negative infections, consistent with lower CD8 T cell selection pressure in HBeAg-positive HBV infection.

Taken together, the CD8 T cell response strongly contributes to HBV sequence diversity, with the core protein being a predominant target of selection pressure. Importantly, viral adaptation to HLA class I-associated selection pressure correlates with markers of viral replication.

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