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Pathophysiology and translational opportunities

Abstract book

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Content

POSTER ABSTRACT PRESENTATIONS - Clinical Science

ORAL ABSTRACT PRESENTATIONS

OS-1-YI
Targeting epithelia-matrix interactions halts cyst growth in polycystic liver disease

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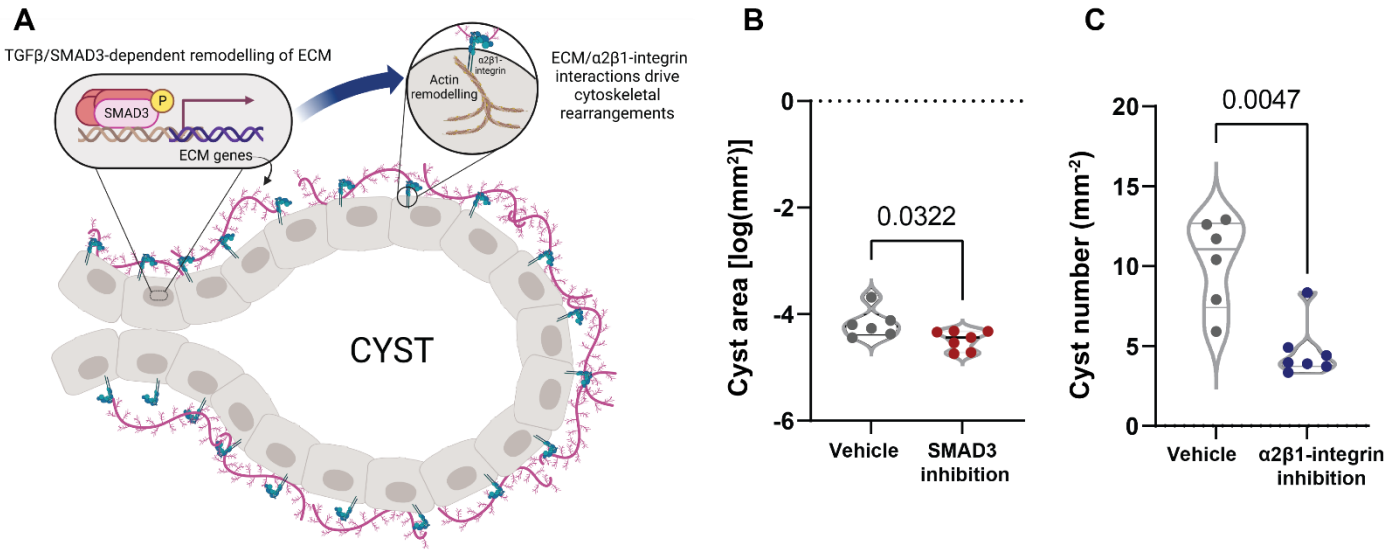
Background and aims: Polycystic liver diseases (PLDs) are a group of genetically heterogeneous pathologies involving the development of large, fluid-filled cysts throughout the liver which result in a poor quality of life for patients. There are very few pharmacological interventions available for PLD patients and none are curative. There is therefore an urgent need to understand the molecular biology underpinning cyst growth in order to develop new therapeutic approaches that could limit cyst growth in PLD. Primary cilia (PC) are sensory organelles and their dysfunction drives cysts in both the liver and kidneys. Little is known as to how PC dysfunction impacts normal cholangiocyte biology in the context of PLD and research into this area holds significant potential to identify candidate targets for therapeutic intervention in PLD.

Method: We generated a novel mouse model of PLD through conditional deletion of the essential PC gene *Wdr35* in biliary epithelial cells (*Krt19$^{CRE-ERT}$; Wdr35$^{-/-}$*) and characterised tissues at 6 and 12 months following PC deletion. Cholangiocytes isolated from *Wdr35$^{+/+}$* and *Wdr35$^{-/-}$* animals at 12 months were subject to single cell RNA sequencing (scRNAseq) to profile the transcriptomes of cystic epithelia. Analysis from scRNAseq and subsequent immunohistochemical validation (IHC) identified candidate targets for pharmacological studies *in vitro* and *in vivo,* namely SMAD3 and α2β1-integrin with the small molecules SIS3 and TC-I 15, respectively.

Results: *Krt19$^{CRE-ERT}$; Wdr35$^{-/-}$* animals show hepatic cysts at 6 months following PC ablation, which grow in number and size until 12 months. Transcriptomic and immunohistochemical profiling of these cystic tissues demonstrates the establishment of a cell-autonomous TGFβ/SMAD3 signalling loop that drives the deposition of extracellular matrix (ECM) around cysts. This remodelled ECM is perceived by α2β1-integrin heterodimers, exclusively expressed on cystic epithelia following PC loss and not on normal cholangiocytes in both rodent and human tissues. Therapeutic targeting of SMAD3 or α2β1-integrin to inhibit cell-matrix interactions in human cystic cholangiocyte lines and *Krt19$^{CRE-ERT}$; Wdr35$^{-/-}$* animals significantly halted cyst growth: SMAD3 inhibition reduced ECM remodeling and consequently cyst size while α2β1-integrin inhibition reduced actin cytoskeletal remodelling and cyst number.

Conclusion: Here, we have combined human PLD tissues with a novel *in vivo* mouse model of PLD to identify common molecular drivers of this disease. We have found that a recurrent TGFβ-ECM-Integrin signalling axis is specifically activated in PLD (**A**) and not healthy biliary epithelial cells and this pathway drives the morphological changes seen as PLD progresses. Importantly, using two small molecule inhibitors of SMAD (**B**) and Integrins (**C**) we are able to limit the progression of PLD *in vivo*.

Figure:


A
TGFβ/SMAD3-dependent remodelling of ECM
SMAD3
P
ECM genes
Actin remodelling
α2β1-integrin
ECM/α2β1-integrin interactions drive cytoskeletal rearrangements
CYST
B
Cyst area [log(mm²)]
0
-2
-4
-6
0.0322
Vehicle
SMAD3 inhibition
C
Cyst number (mm⁻²)
20
15
10
5
0
0.0047
Vehicle
α2β1-integrin inhibition

OS-2-YI
Hepatic single-cell transcriptome in primary sclerosing cholangitis

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Background and aims: Primary sclerosing cholangitis (PSC) is a rare liver disease characterised by cholangitis and periportal fibrosis resulting in bile duct sclerosis. Molecular mechanisms and cell-cell interactions driving PSC progression are largely unknown. We aimed to assess the molecular interactome in a single-cell dataset from a human PSC liver and performed in silico ligand screening.

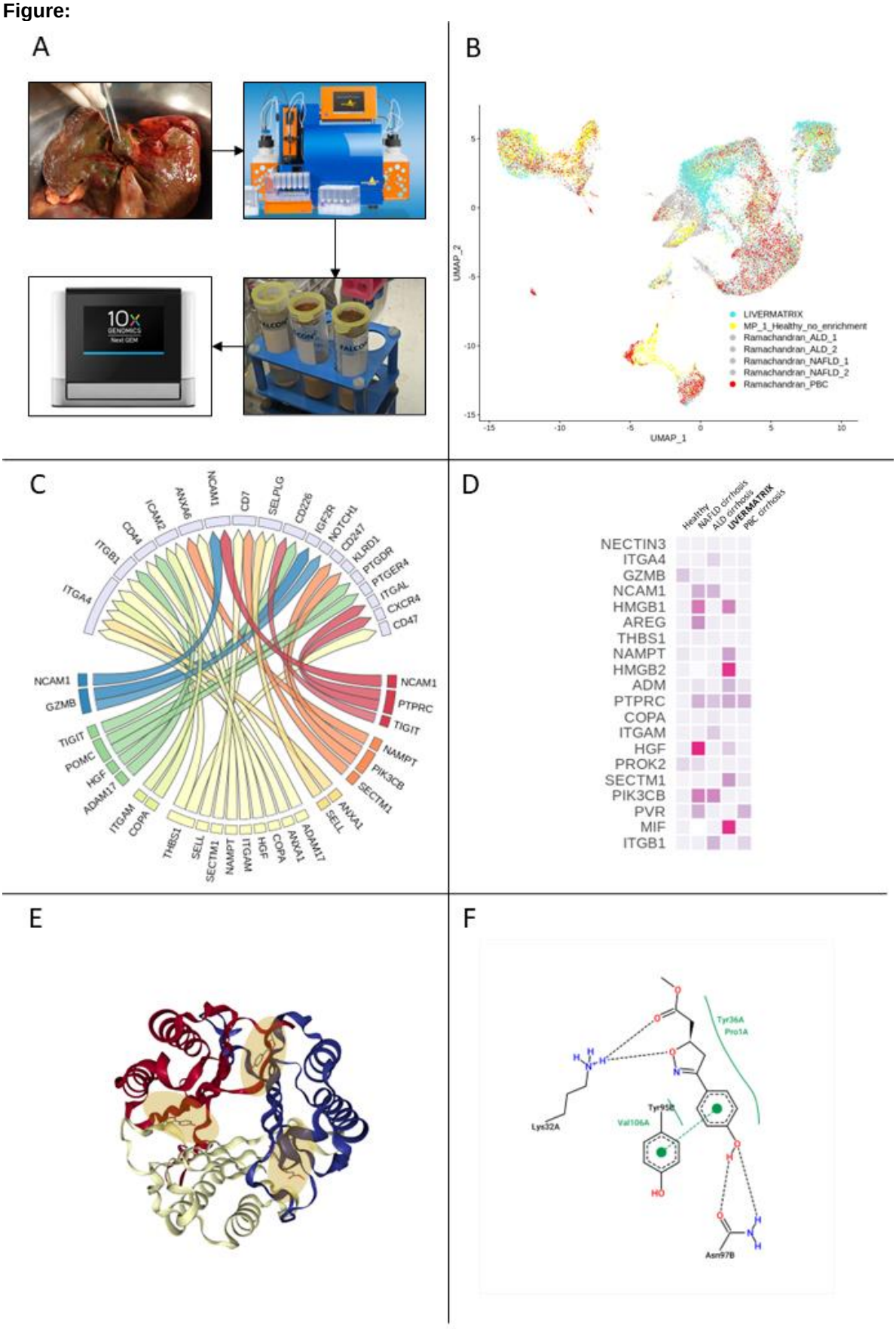
Method: A patient with PSC enrolled in the LIVERMATRIX study (MUV EC 2318/2019) underwent liver transplantation. Primary hepatic cells were isolated from the explanted liver by mechanical and enzymatic (0.13 WU/ml Liberase) dissociation of liver tissue. Total non-parenchymal cells (NPC) were separated by differential centrifugation, cryopreserved, and sequenced using the 10x single-cell RNA (scRNA-seq) protocol. The obtained dataset was integrated with healthy and cirrhotic liver scRNA-seq datasets (MacParland et al. 2018; Ramachandran et al. 2019) for the reference annotation using seurat. With CellChat, we predicted cell interactions in the PSC dataset based on known ligand-receptor pairs and identified best-matching cell populations. These cell populations were then subjected to differential interaction analysis versus other aetiologies with NicheNet. Targets specific to PSC were used for ligand library construction. Finally, ligands were ranked based on molecular docking using AutoDock Vina.

Results: NPC sequencing yielded 8868 cells post-quality control from the human PSC liver sample. Integration with reference datasets confirmed representation of the sequenced PSC cells in all reference clusters, with enrichment of activated γδ-T-cells and depletion of tolerogenic *MARCO*+ Kupffer cells (KC). Cell interaction analysis showed high activity of fibrogenic *ITGB2*, *ADGRE5*, *GALECTIN* pathways, with activated KC and γδ-T-cells playing a key source of profibrogenic signalling. In differential interaction analysis with other immune cells, the isolated cells were enriched for *MIF*, *HMGB2* and *HMGB1*. Markers overrepresented in PSC were subjected to molecular docking, which prioritised both known (e.g., ISO-1 for MIF) and previously undescribed inhibitors (e.g., regorafenib for *ITGB2*) as potential ligands in PSC.

Conclusion: Single-cell interactome in PSC involves the activation of γδ-T-cells and KC populations as well as the depletion of tolerogenic cells. Their most enriched molecular signals relate to cell adhesion and fibrogenesis. This differential interactome analysis identified potential PSC-specific molecular targets with their ligands that should be further investigated in vitro and in vivo experiments.

We have submitted two additional paired single-cell and single-nuclei RNA sequencing samples from explanted livers of patients diagnosed with PSC. We expect to obtain the data and perform additional analysis to present the results at the conference.

Figure:


A
10x GENOMICS
B
UMAP_2
UMAP_1
LIVERMATRIX
MP_1_Healthy_no_enrichment
Ramachandran_ALD_1
Ramachandran_ALD_2
Ramachandran_NAFLD_1
Ramachandran_NAFLD_2
Ramachandran_PBC
C
NCAM1
CD7
SELPLG
CD226
IGF2R
NOTCH1
CD247
KLRD1
PTGDR
PTGER4
ITGAL
CXCR4
CD47
ITGA4
ITGB1
CD44
ICAM2
ANXA6
GZMB
TIGIT
POMC
HGF
ADAM17
ITGAM
COPA
THBS1
SELL
SECTM1
NAMPT
PTPRC
PIK3CB
ANXA1
ADAM17
D
Healthy
NAFLD cirrhosis
ALD cirrhosis
LIVERMATRIX
PBC cirrhosis
NECTIN3
ITGA4
GZMB
NCAM1
HMGB1
AREG
THBS1
NAMPT
HMGB2
ADM
PTPRC
COPA
ITGAM
HGF
PROK2
SECTM1
PIK3CB
PVR
MIF
ITGB1
E
F
Tyr36A
Pro1A
Lys32A
Tyr95B
Val106A
Asn97B

OS-3
Dual alpha-v/beta-6 and alpha-v/beta-1 integrin inhibitors reduce TGF-beta signaling and fibrosis in multiple preclinical rodent models of biliary fibrosis

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Background and aims: Integrins alpha-v/beta-6 and alpha-v/beta-1 are heterodimeric cell-surface proteins that bind to and activate latent transforming growth factor (TGF)-beta, a key driver of fibrosis. In primary sclerosing cholangitis (PSC), integrins alpha-v/beta-6 and alpha-v/beta-1 are thought to play a role in the development and propagation of fibrosis through the activation of TGF-beta by cholangiocytes and myofibroblasts, respectively. Bexotegrast (PLN-74809) is a dual inhibitor of TGF-beta-activating integrins alpha-v/beta-6 and alpha-v/beta-1 currently in clinical development for the treatment of PSC. Here we demonstrate the ability of bexotegrast and additional dual alpha-v/beta-6 and alpha-v/beta-1 inhibitors, with similar profiles, to block biliary fibrosis in three different rodent models of biliary fibrosis.

Method: Biliary fibrosis was induced in mice by homozygous deletion of the Mdr2 gene (Mdr2-/-) or 3, 5-diethoxycarbonyl-1, 4-dihydrocollidine (DDC) diet-feeding, and in rats through bile duct ligation (BDL). Animals were treated with either bexotegrast or tool alpha-v/beta-6 and alpha-v/beta-1 inhibitors, PLN-75068 or PLN-169. Readouts included hepatic SMAD2/3 phosphorylation, hepatic hydroxyproline concentration, picrosirius red (PSR) staining for collagen, and serum alkaline phosphatase (ALP) levels. Differences with p values less than 0.05 were considered significant.

Results: In fibrotic Mdr2-/- mice, bexotegrast significantly reduced hepatic collagen (~25% hydroxyproline; 50% collagen proportional area [CPA]) and ALP levels (46%). In agreement with a mechanism of action targeting TGF-beta signaling, bexotegrast reduced hepatic SMAD phosphorylation (42%). PLN-75068 behaved similarly in an independent Mdr2-/- study. In fibrotic DDC-fed mice, PLN-75068 also significantly reduced hepatic collagen (23% hydroxyproline) and ALP levels (49%). In BDL rats, PLN-169 significantly reduced hepatic SMAD phosphorylation (55%) and hepatic collagen (~50% CPA).

Conclusion: In three independent models of biliary fibrosis, dual inhibition of alpha-v/beta-6 and alpha-v/beta-1 inhibited TGF-beta signaling and reduced hepatic fibrosis. This highlights the important role of these integrins in driving fibrogenesis associated with biliary injury. Bexotegrast is currently being evaluated in the INTEGRIS-PSC phase 2a study in participants with PSC (EudraCT: 2020-001428-33, NCT04480840).

OS-4-YI
Immuno-oncology biomarkers for improved diagnosis of cholangiocarcinoma in primary sclerosing cholangitis: insights from the inflammatory and fibrotic tumour microenvironment

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Background and aims: Cholangiocarcinoma (CCA) is a rare but highly aggressive malignancy that arises from the epithelial cells of the biliary tree. Unfortunately, CCA is often asymptomatic in its early stages, making it difficult to diagnose and treat effectively. As a result, CCA patients have a poor prognosis (5-year survival <10%). Patients with underlying primary sclerosing cholangitis (PSC), a chronic autoimmune disease that causes inflammation and fibrosis of the bile ducts, are at increased risk of developing CCA, making them candidates for monitoring. However, there are no reliable markers to predict progression from PSC to CCA. Our aim was to identify potential immuno-oncology biomarkers that can improve the early diagnosis of CCA in patients with PSC and determine whether some biomarkers could also be useful for the diagnosis of patients with CCA of other aetiologies.

Method: A proteomic analysis was performed on serum samples from 35 PSC, 35 sporadic CCA, 12 CCA patients with a background of PSC, and 20 healthy controls using the Olink® Explore 384-plex inflammation and oncology panels and Luminex ProcartaPlex immune-monitoring assays. The resulting serum protein signatures were analysed to discriminate PSC from CCA cases, as well as PSC-CCA from PSC and PSC-CCA from CCA patients. The biological relevance of these markers was further explored using spatial proteomics (Nanostring GeoMx platform) on representative tissue sections from each group of comparison.

Results: Several serum protein signatures were identified that could distinguish PSC from CCA patients, and also PSC-CCA from PSC and from sporadic CCA patients. The identified immuno-oncology biomarkers included proteins involved in immune system regulation, extracellular matrix remodelling, and cell growth and differentiation. Spatially-resolved proteomic analysis confirmed a dysregulation of the inflammatory and fibrotic tumour microenvironment of PSC patients that developed CCA.

Conclusion: The identification of immuno-oncology biomarkers that can differentiate PSC from CCA patients and PSC-CCA from PSC or CCA patients is an important step towards improving the early diagnosis of CCA in PSC. These biomarkers provide insights into the complex interplay between inflammation and fibrosis in the tumour microenvironment and have the potential to be used as early diagnostic and prognostic markers for CCA in PSC patients.

Figure:

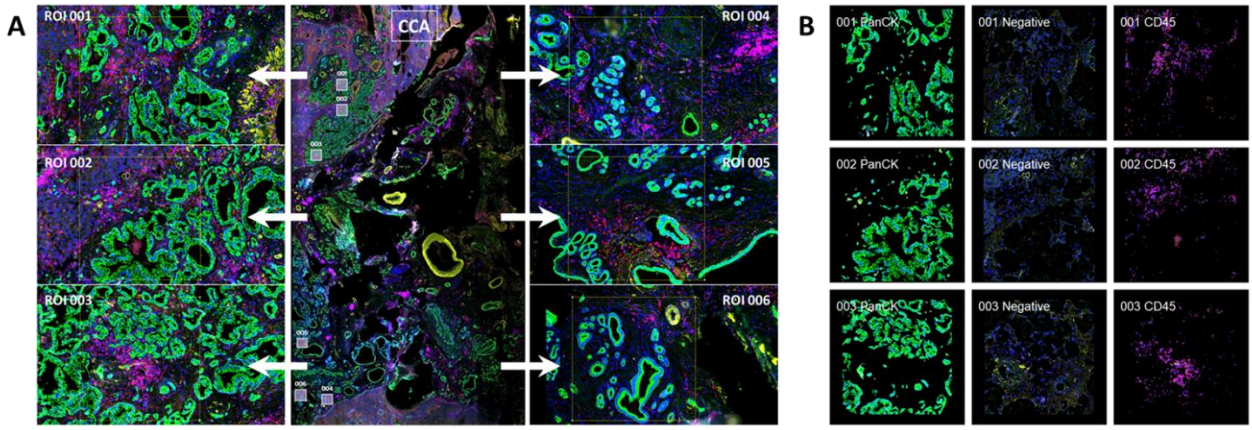

Figure 1. Region of interest (ROI) selection and segmentation strategy performed for spatial transcriptomics (GeoMx platform). A) Representative images from the ROI selection in CCA. PanCK+ (green), CD45+ (pink), αSMA (yellow) and nuclei counterstained in blue. **B)** Segmentation strategy. The tissue area of each ROI was further divided into PanCK+, CD45+ and PanCK-CD45- subsegments (3 representative ROIs shown).

OS-5
Osteopontin prevents excessive macrophage-mediated bile duct injury in experimental sclerosing cholangitis and correlates with liver injury in human patients

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Background and aims: Primary sclerosing cholangitis (PSC) is an immune-mediated cholestatic liver disease for which pharmacological treatment options are currently unavailable. PSC is strongly associated with colitis and a disruption of the gut-liver axis, and macrophages are involved in the pathogenesis of PSC. However, how gut-liver interactions and specific macrophage populations contribute to PSC is incompletely understood.

Method: We investigated the impact of cholestasis and colitis on hepatic and colonic inflammation, and performed an in depth characterization of hepatic macrophage dynamics and function in models of concomitant cholangitis and colitis. Additionally, we provided translational relevance by extending our findings to PSC patients.

Results: Cholestasis-induced fibrosis was characterized by depletion of Kupffer cells, and enrichment of monocytes and monocyte-derived macrophages (MoMFs) in the liver. These MoMFs highly express triggering receptor expressed on myeloid cells 2 (Trem2) and osteopontin (Spp1), markers assigned to hepatic bile duct associated macrophages, and were enriched around the portal triad, which was confirmed in human PSC. Colitis induced monocyte/macrophage infiltration in the gut and liver, enhanced cholestasis-induced MoMF-Trem2 and Spp1 upregulation, yet did not exacerbate liver fibrosis. Bone marrow chimeras showed that knockout of Spp1 in infiltrated MoMF exacerbates liver inflammation, which was confirmed in vitro. In human patients serum sTREM2 and osteopontin levels are elevated and correlate with liver injury.

Conclusion: Our data shed light on the mechanisms involved in gut-liver axis perturbations and macrophage dynamics and function in obstructive fibrosing cholangitis and colitis, highlight Spp1 as important macrophage regulator in disease progression and indicate its potential as future therapeutic target in PSC.

OS-6-YI
The planar cell polarity pathway as a master regulator of terminal biliary morphogenesis during liver development

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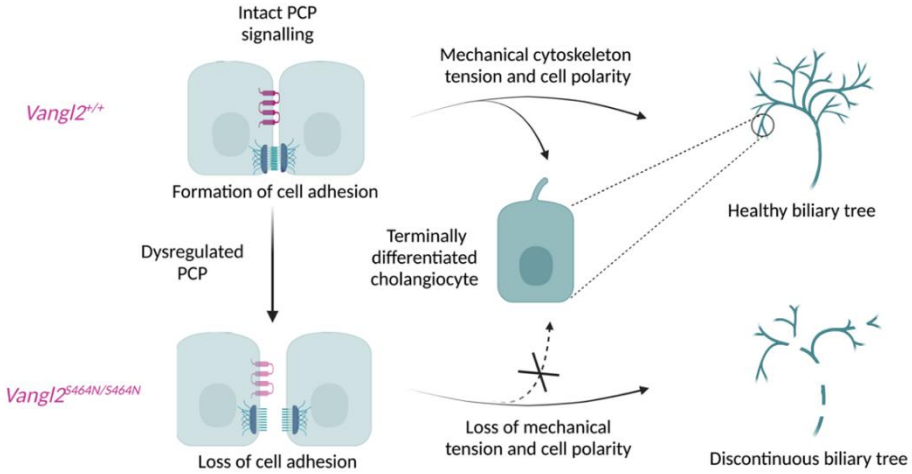
Background and aims: The biliary tree is a complex ductular network within the liver that transports bile to the intestine. The formation of a functional biliary system during development is indispensable to mammalian health and aberrant bile duct formation leads to cholestatic diseases, such as biliary atresia. While human genetic diseases, such as Alagille syndrome and animal studies have helped to identify the master regulators of the biliary cell lineage including Notch and TGFβ signalling, little is known about the mechanisms that drive the transformation of a relatively simple ductal plate into a complex tubular network. Here, we aimed to identify candidate regulators of terminal morphogenesis in bile duct development and functionally define the molecular processes that underpin this process.

Method: Using single-cell RNA sequencing of embryonic mouse tissue we define the molecular regulators of terminal ductular morphogenesis. We then combine transgenic and mutant mouse lines with 3D whole-mount immunofluorescence to define functionally the processes by which ducts undergo morphogenesis. Finally, we make use of 3D organoid cultures to model how defects in ductular morphogenesis arise and use unbiased mass spectroscopy and proteomics to identify key regulators in ductular morphogenesis.

Results: In embryonic mouse livers, the transcriptional expression of Planar Cell Polarity (PCP) pathway components increases between embryonic stages E14.5 and E18.5, during which the ductal plate undergoes large-scale morphological rearrangement. Throughout ductular morphogenesis, VANGL2, a core PCP component, localises to cell-cell junctions of ductular cells and interacts with desmosome proteins, suggesting a novel role for VANGL2 in supporting desmosome function and normal mechanical patterning of the developing duct. Consequently, the genetic loss of *Vangl2* function results in a failure to establish a continuous biliary network and mutant animals display fewer ductular branches and iterative gaps across the biliary tree. Additionally, mutant bile ducts display aberrant apical-basal cell polarity as evidenced by: (i) disrupted cytoskeletal arrangements, and (ii) impaired efflux pump activity, thus highlighting the essential link between normal tissue morphology and bile duct physiology.

Conclusion: Using a combination of *in vitro* and *in vivo* systems, we demonstrate that planar cell polarity signaling in the developing bile ducts, is essential for the normal terminal development of the biliary tree. Through characterisation of the consequences to cell biology following loss of PCP, we found that PCP underpins normal morphogenesis by establishing cytoskeletal networks thereby supporting cell junction integrity and ductular elongation, providing one of the first mammalian descriptions of the biomechanical regulators of bile duct morphogenesis.

Figure:



Schematic of the role of PCP signaling and VANGL2 in biliary development. In VANGL2$^{+/+}$ biliary epithelial cells (BECs), PCP signaling induces cell polarity which results in mechanical tension along the developing ducts. This coincides with the process of terminal differentiation leading to a healthy and continuous bile duct network. In VANGL2$^{S464N/S464N}$ mice, dysregulated PCP leads to improperly formed cell adhesion between BECs. As a consequence, bile duct cells lose cell polarity and mechanical tension and fail to undergo terminal differentiation leading to a discontinuous biliary tree.

OS-7-YI
Liver transcriptome analysis reveals PSC-specific gene signature in biliary fibrosis

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Background and aims: Primary Sclerosing Cholangitis (PSC) is a chronic heterogenous cholangiopathy with unknown aetiology where chronic inflammation leads to cholestasis, multifocal biliary strictures and fibrosis with consecutive cirrhosis development. We here aimed to identify a PSC-specific gene signature associated with biliary fibrosis development.

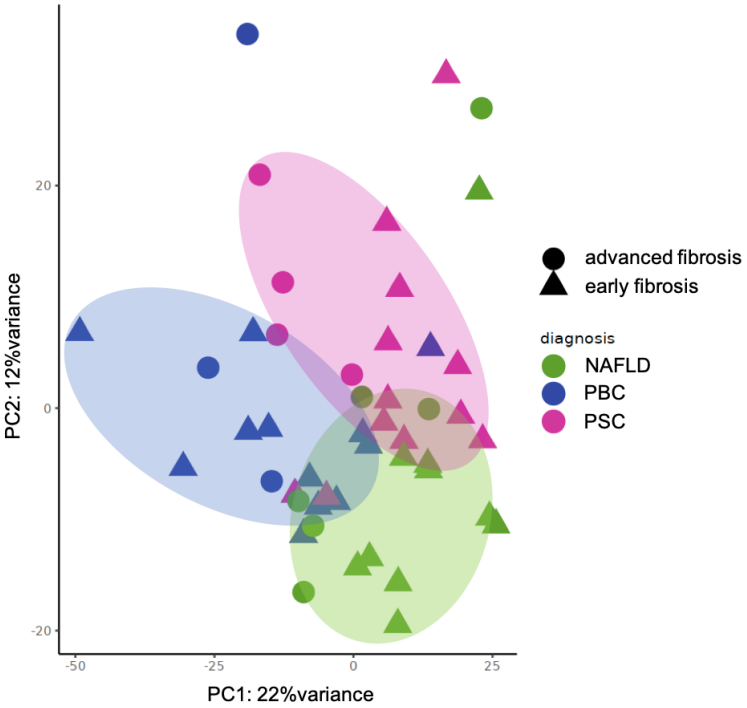
Method: In our study, we undertook RNA-sequencing of 47 liver biopsies from patients with PSC (n = 16), primary biliary cholangitis (PBC, n = 15), and non-alcoholic fatty liver disease (NAFLD, n = 16) with different fibrosis stages to identify a PSC-specific gene signature associated with biliary fibrosis development. For validation, we compared an external transcriptome data set of liver biopsies from PSC patients (n = 73) with different fibrosis stages (DOI: 10.1002/hep.31488; baseline samples from NCT01672853). To further investigate advanced fibrosis in PSC, explanted liver tissue from patients with PSC cirrhosis (n = 6), alcohol-related cirrhosis (n = 5) and controls without chronic liver disease (n = 4) was available for RNA sequencing.

Results: Differential gene expression analysis of the liver transcriptome from PSC patients with advanced versus early fibrosis revealed 431 genes associated with fibrosis development. Of those, 376 were identified as PSC-specific fibrosis-associated genes after comparison to pro-fibrotic gene signatures in liver biopsies from patients with PBC or NAFLD. After equivalent analysis of an external cohort of 73 liver biopsies from PSC patients with different fibrosis stages, 127 genes were found to be differentially expressed between advanced versus early PSC fibrosis in both data sets independently. Further investigation of advanced (biliary) fibrosis by comparison of the liver transcriptome from patients with PSC cirrhosis to either alcohol-related cirrhosis or controls without chronic liver disease underlined the strong etiology-specific impact on fibrosis-related genes. Finally, 10 genes in all three data sets were found to be PSC-specific and pro-fibrogenic (CCDC80, COL1A1, COL1A2, COMP, KRT81, MFAP4, PLAT, RERG, SFRP4, SLCO4C1, TMEM200A), indicating a pivotal role in the development of PSC-specific biliary fibrosis.

Conclusion: We reveal a PSC-specific gene signature associated with biliary fibrosis development that may enable the identification of potential new biomarkers and therapeutic targets in PSC fibrosis.

Figure:

Liver transcriptome analysis reveals disease-specific fibrosis-dependent clustering in PSC, PBC and NAFLD. Principal component analysis (PCA) after RNA-sequencing of liver samples of patients with primary sclerosing cholangitis (PSC, n = 16), primary biliary cholangitis (PBC, n = 15), and non-alcoholic fatty liver disease (NAFLD, n = 16) subdivided into advanced and early fibrosis (3-4/4 and 0-2/4). Corrected counts of the filtered genes are used.



OS-8
Quantitative MRCP metric to distinguish IgG4-sclerosing cholangitis from primary sclerosing cholangitis

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Background and aims: Immunoglobulin G4-related sclerosing cholangitis (IgG4-SC) is often difficult to distinguish from primary sclerosing cholangitis (PSC) using traditional imaging assessments. We hypothesise that quantitative biliary tree assessments would enable stratification of patients with PSC and IgG4-SC.

Method: We recruited patients with histologically-confirmed IgG4-related disease. 3D MRCP data were acquired at 1.5T and processed using MRCP+ (Perspectum, Oxford, UK). Age- and sex-matched large-duct PSC controls were selected from a previous study. MRCP+ metrics with good or excellent scan-rescan repeatability (ICC >0.60) were included in analysis. The diagnostic performance of MRCP+ metrics with statistically significant differences between PSC and IgG4-SC, as well as serum IgG4 at a previously published threshold (>2.8 g/L) were examined using receiver operating characteristic curve analyses. Area under the curve (AUC), sensitivity and specificity were recorded at the cut-off point that maximised the Youden index. Data is presented as median (range).

Results: Twelve patients were recruited (6 male, median age 67 [46-79] years, disease duration: 2 [1-9] years). The main disease phenotype was pancreatobiliary involvement (n = 10) with 9 patients having active disease at recruitment. MRCP+ analysis was successful in 11 patients. One patient had MRCP+ pre and post-treatment with steroids (Figure 1). Median serum IgG4 was elevated in patients with IgG4-SC compared to PSC (IgG4-RD vs PSC: 2.29 [0.05-13.14] vs 0.32 [0.08-0.84] g/L, p = 0.001). It had 100% specificity for detecting patients with IgG4-SC but lower sensitivity (57%) at 2.8 g/L threshold. The percentage of ducts with median diameter 3-5 mm was lower in IgG4-SC than PSC (16 [11-28] vs 32 [21-36]; p = 0.03). A cut-off value of 16.4% was able to differentiate the two conditions with AUC of 0.81 (0.58-1.00). Using serum IgG4 as a first line assessment and the percentage of ducts with median diameter 3-5 mm as a second line assessment in patients with low serum IgG4, maintained 100% specificity observed using serum IgG4 alone but increased the sensitivity of detection in this small cohort to 71%.

Conclusion: Quantitative MRCP metric may aid the differentiation of patients with IgG4-SC from those with PSC. The percentage of ducts with median diameter 3-5 mm has previously been shown to predict disease severity and transplant-free survival in patients with PSC, but further suitably powered study is needed to test its discriminatory value in differentiating IgG4-SC from PSC.

Figure:

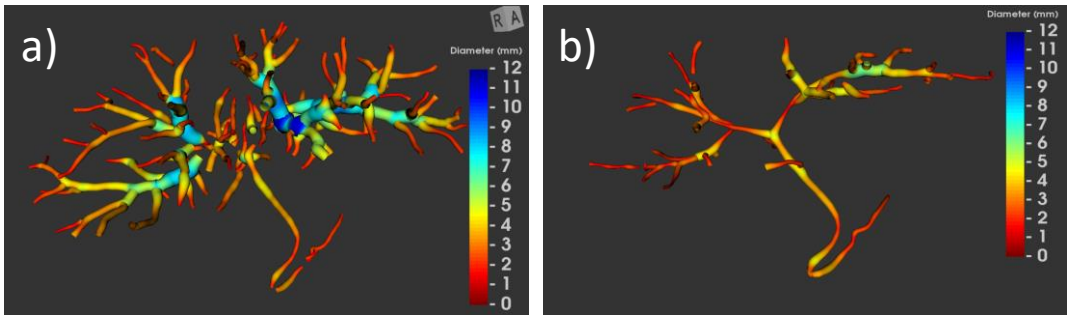

Figure 1: MRCP+-derived biliary tree model in a patient with IgG4-related sclerosing cholangitis a) pre- and; b) post treatment with prednisolone therapy. The percentage of ducts with median diameter 3-5mm reduced from 39% to 26%.

POSTER ABSTRACT PRESENTATIONS

Basic Science

P01-01-YI
Acute hepatocytic loss of endosomal sorting complex Retromer increases proliferation of biliary epithelial cells (BECs)

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Background and aims: Ductular reaction (DR) is a phenomenon observed in various biliary disorders, including primary biliary cholangitis. DR is characterized by increased proliferation of biliary epithelial cells (BECs/cholangiocytes), portal inflammation and fibrosis. It has been shown that during DR, cholangiocytes can transdifferentiate into hepatocytes. However, how intercellular communication is regulated to orchestrate DR remains unclear. Here we report a new mouse model to study the underlying mechanism of DR.

Method: Using somatic CRISPR/Cas9-mediated gene editing we ablated *Vps35*, a component of an endosomal sorting complex, in hepatocytes of adult mice. 8 (short) and 26 (long) weeks after VPS35 inactivation, hepatocellular proliferation, fibrosis, apoptosis, and DR were determined by histological staining for BrdU and Ki67, sirius red, cleaved caspase 3 and CK19, respectively. Furthermore, liver inflammation was assessed by immunohistochemical staining for CD11b, F4/80, and CD3, together with gene expression analyses for *Tnf*, *Il6* and *Il1b*. Plasma liver enzymes (ALT and AST) and bile acid levels were measured.

Results: Acute hepatocytic loss of VPS35 resulted in increased BEC proliferation and fibrosis, accompanied by infiltration of $CD3^+$ T cells into the portal tracts and increased plasma ALT and AST levels, without an increase in the number of cleaved caspase 3 positive cells. Plasma bile acids were only increased in mice with short term VPS35 ablation but not in mice with long term VPS35 inactivity. Gene expression of oval cell and progenitor markers *Cd24a* and *Ck18* were increased, while *Alb* (albumin) and *Hnf4a* expression were decreased. 26 weeks after VPS35 ablation, hepatocellular proliferation and plasma ALT and AST levels were still increased, without a sign of fibrosis. Interestingly, hepatic VPS35 protein levels were markedly higher in mice with long term VPS35 ablation compared to mice with short term ablation.

Conclusion: We present a novel mouse model to study the mechanisms underlying DR and show for the first time that impaired Retromer function leads to all characteristics of DR without severe liver damage. We hypothesize that acute loss of hepatocytic VPS35 activates BEC proliferation and trans-differentiation of BECs into hepatocytes through a signalling pathway that has yet to be identified.

P01-02
An organotypic model of ductular reaction reveals a role of lipid biosynthesis in biliary cell activation

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Background and aims: The adult liver has a slow homeostatic turnover rate, but a remarkable ability to regenerate following damage. In response to hepatocyte injury, biliary epithelial cells undergo reprogramming into liver progenitors and expand to repopulate the liver parenchyma, a regenerative process known as ductular reaction. However, owing on its pro-inflammatory and fibrogenic capacity, chronic ductular reaction has been linked to liver disease progression, making it an attractive therapeutic target. The molecular dynamics coordinating the transition of biliary epithelial cells from quiescence to a proliferative state are largely unknown, but cell-autonomous mechanisms and crosstalk with the physical microenvironment are thought to be involved.

Method: In this study, we report the generation of a novel culture system able to recapitulate a ductular reaction, ex vivo, while maintaining the mechanical properties of microenvironment as native. This system is established by culturing precision-cut liver slices in a defined medium supplemented with a number of specific growth factors.

Results: Using both this platform and biliary organoids derived from mouse and human livers, we reveal that the mevalonate pathway is activated in liver progenitors and that this metabolic signal is instrumental to establish a ductular reaction response. Mechanistically, we found that mevalonate-derived bioproducts fuel liver progenitor proliferation via activating the MAPK signaling.

Conclusion: This novel culture system represents a valuable tool for the study of liver regeneration in vitro and suggests the use of statins as a therapeutic option to prevent chronic liver diseases progression.

P01-04
Characterizing and manipulating GPR35-dependent mast cell activity in PSC

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Background and aims: Mast cells are associated with tissue fibrosis in a variety of chronic inflammatory conditions, including fibrotic liver disease. We observed infiltration of mast cells in a subset of cirrhotic explant livers of patients with primary sclerosing cholangitis (PSC) that expressed the orphan G protein-coupled receptor 35 (GPR35). GPR35 is a major genetic risk factor for PSC and inflammatory bowel disease (IBD). Consequently, we aimed to delineate the role of GPR35-dependent mast cell activity during biliary fibrosis in PSC.

Method: We used mast cell markers tryptase, chymase, c-KIT, MRGX2 and immunofluorescence to quantify mast cells in explant liver tissue from patients diagnosed with PSC (n = 10), primary biliary cholangitis (PBC, n = 3) and alcohol-related liver disease (ARLD, n = 3). To evaluate GPR35-dependent mast cell biology, we generated *GPR35* knock-out HMC-1 and LUVA human mast cell lines using CRISPR/Cas9. GPR35-dependent signalling was assessed using chimeric G protein- and Ca^{2+} flux assays. Mast cell activation and degranulation was measured using IL-4, IL-5 and IL-13 ELISA, qRT-PCR and beta-hexosaminidase assay.

Results: GPR35-positive mast cells were detected in fibrotic regions around bile ducts and were absent in parenchymal liver tissue. Compared to PBC and ARLD, mast cell numbers were significantly increased in PSC liver tissue and localized to the common bile duct. We detected no IgE-positive B cells in PSC livers using single-cell RNA sequencing (n = 3) and no IgE in culture supernatants of mononuclear cells from PSC explants (n = 30). IL-4, IL-5 and IL-13 expression by qRT-PCR was significantly higher in wild-type cells compared to *GPR35* knock-outs. Mast cell degranulation of wild-type cells was strongly inhibited by treatment with the GPR35 ligands cromolyn and lodoxamide whilst minimal effect was observed with the *GPR35* knock-outs.

Conclusion: These findings imply IgE-independent mast cell activation in PSC pathogenesis. *In vitro* experiments suggest that IgE-dependent mast cell activity and degranulation are modulated by GPR35, promoting secretion of pro-fibrotic mediators. As a result, we propose a novel role for the PSC and IBD risk gene *GPR35* in mast cell biology particularly relevant in chronic inflammatory and fibrotic disease.

P01-05-YI
Necroptosis contributes to liver inflammation and fibrosis in experimental primary sclerosing cholangitis

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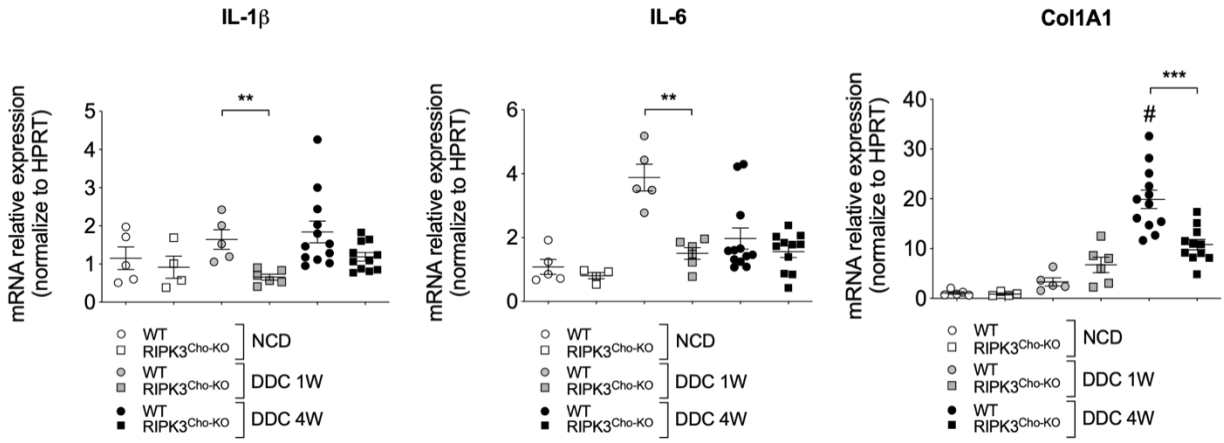
Background and aims: Necroptosis is a regulated and proinflammatory cell death pathway, involved in many liver diseases. There is evidence that cholangiocytes, under basal conditions and in an experimental model of cholangiopathy, express the necroptotic effector kinase RIPK3 at high levels. The aim of our project is to determine the contribution of necroptosis to the pathogenesis of primary sclerosing cholangitis (PSC).

Method: We developed a murine line with a specific knockout (KO) of RIPK3 in cholangiocytes ($RIPK3^{Cho\text{-}KO}$). We used a xenobiotic model of sclerosing cholangitis based on 3, 5-diethoxycarbonyl-1, 4-dihydro-collidine (DDC) feeding. Inflammatory and fibrosis markers were analyzed on whole liver by reverse transcription quantitative PCR (RT-qPCR) at two end points: after 1 week of DDC feeding, corresponding to the early inflammatory phase of cholangitis, and after 4 weeks of DDC feeding, corresponding to the fibrosing phase of cholangitis.

Results: RIPK3 KO in cholangiocytes was confirmed after a collagenase-pronase extraction of the biliary tree. After 1 week of DDC feeding, expression of inflammatory markers (*Interleukin-1β* and *Interleukin-6*) was lower in the KO mice compared with wild-type (WT) mice. After 4 weeks of DDC feeding, expression of *Collagen 1A1* in the KO mice liver was lower compared with wild-type mice.

Conclusion: These data suggest that cholangiocytes necroptosis is deeply involved in the promotion of inflammation and fibrosis in sclerosing cholangitis. Additional studies on liver and sera from PSC patients are currently underway to confirm these preliminary results.

Figure: *RT-qPCR analysis of IL-1beta, IL-6 and Col1a1 expression in the liver of tamoxifen-treated Osteopontin-CreER^{T2};RIPK3$^{loxP/loxP}$ mice (RIPK3$^{Cho\text{-}KO}$), shown in comparison with wild type (WT). The mice were fed either normal chow diet (NCD) or 3, 5-diethoxycarbonyl-1, 4-dihydro-collidine (DDC) as indicated.*



P01-06-YI
Multimodal decoding of the mesenchymal landscape of human biliary fibrosis

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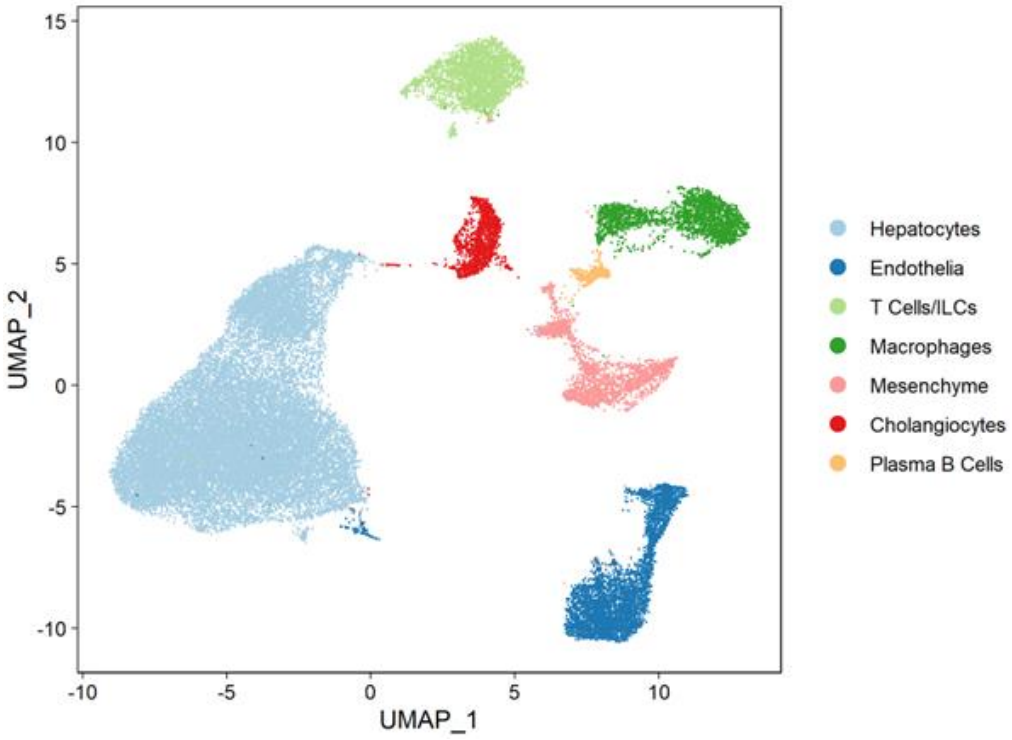
Background and aims: Primary Biliary Cholangitis (PBC) is a fibrosing cholangiopathy of the liver that is increasing in prevalence globally. Currently, there are no effective antifibrotic therapies available with which to treat patients with liver fibrosis. In end-stage disease, transplantation is the only treatment option, however, less than 10% of global transplantation needs are met. Therefore, effective antifibrotic therapies are urgently required. To advance our understanding of human liver fibrosis and to inform design of antifibrotic therapies, we have used multimodal single cell genomics approaches to generate single-cell, pan-lineage atlases of human primary biliary cholangitis and mouse models of biliary fibrosis.

Method: To deepen our understanding of the cellular and molecular mechanisms driving human biliary fibrosis, and to inform design of effective antifibrotic therapies, we performed single nuclei RNA sequencing (snRNA-seq) of healthy and PBC explant livers to generate a single-cell, multi-lineage atlas of human biliary fibrosis. Following this, we established a single-nuclei pan-lineage atlas of mouse biliary fibrosis and used this mouse model to interrogate the function of corollary subpopulations observed in both human and mouse biliary fibrosis

Results: We uncovered multiple, disease-associated mesenchymal and cholangiocytes subpopulations in human biliary fibrosis and using newly generated markers we have comprehensively characterised the topography of these subpopulations in the human biliary fibrotic niche. Moreover, ligand-receptor analysis of pathogenic mesenchymal subpopulations has identified a distinct set of putative therapeutic targets, which we are currently functionally interrogating using genetic approaches in mouse biliary injury models, and small molecule approaches in human biliary, multi-lineage organoids.

Conclusion: Multimodal single cell genomic approaches, in conjunction with functional interrogation of corollary cellular subpopulations in mouse models of biliary fibrosis, have allowed us to uncover novel cell states and unanticipated aspects of biliary fibrogenesis. Our ongoing work is currently assessing the feasibility and tractability of these newly identified therapeutic targets to treat patients with biliary fibrosis.

Figure: UMAP delineating a single-nuclei pan-lineage atlas of healthy and PBC human liver (n = 10) showing major cell lineage cluster annotations.



P01-07-YI
Silencing CNNM4 in cholangiocarcinoma inhibits tumoral progression by means of non-canonical ferroptosis

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Background and aims: Cholangiocarcinoma (CCA) is a heterogeneous neoplasm of biliary ducts that represents the second most common primary hepatic cancer, after hepatocellular carcinoma. Due to its aggressiveness, late diagnosis and immunoregulatory capacity of the disease, CCA outcomes are poor, with a median overall survival of less than 12 months. Currently, the only curative treatment is surgical resection, but this only applies to 25% of cases and despite this tumoral recurrence is frequent. For that reason, the study of new therapies is of utmost importance. Recent studies show that the isocitrate dehydrogenase 1 (*IDH1*) inhibitor, used to treat patients with irresectable iCCA harboring *IDH1* mutations, reduces cell proliferation, invasion and metastasis by promoting, ferroptosis, a programed cell death caused by iron-dependent lipid peroxidation.

Results: In this study, we analyze the role of CNNM4 (*Cyclin and CBS Domain Divalent Metal Cation Transport Mediator 4*), a Mg^{2+} effluxer, that is overexpressed in CCA in *in silico*, at transcriptional levels and also in human biopsies. Silencing CNNM4 in CCA human cell lines, EGI-1 and TFK-1, which show high expression of CNNM4, not only increases intracellular Mg^{2+} but also reduces cellular proliferation and sensitizes cells to chemotherapeutic drugs. Key metastasis steps (intravasation, extravasation and invasion in other organs) were also slowed down when CNNM4 is silenced, as seen by 3D spheroid experiments and in *ex ovo* and *in ovo* chicken embryo chorioallantoic membrane assay. Proteomic analysis reveals a metabolic shift into a less glycolytic phenotype in CNNM4-silenced cells, also indicating a role of this transporter in the Warburg effect. Alteration of iron metabolism after CNNM4 modulation in both cell lines is associated with a decrease of NUPR-1 levels, a ferroptosis inhibitor, that can be a possible mechanism of those effects. In a CCA murine model (myr-AKT/Yap^{S127A}), silencing CNNM4 after tumoral development, via a liver-specific molecule, produces its reversion.

Conclusion: In conclusion, silencing CNNM4 is a potential therapeutic target in CCA whose effect could be mediated by iron-dependent cell death.

P01-08
Harnessing immune dysregulation in the search for serum biomarkers for diagnosis and prognosis of primary sclerosing cholangitis

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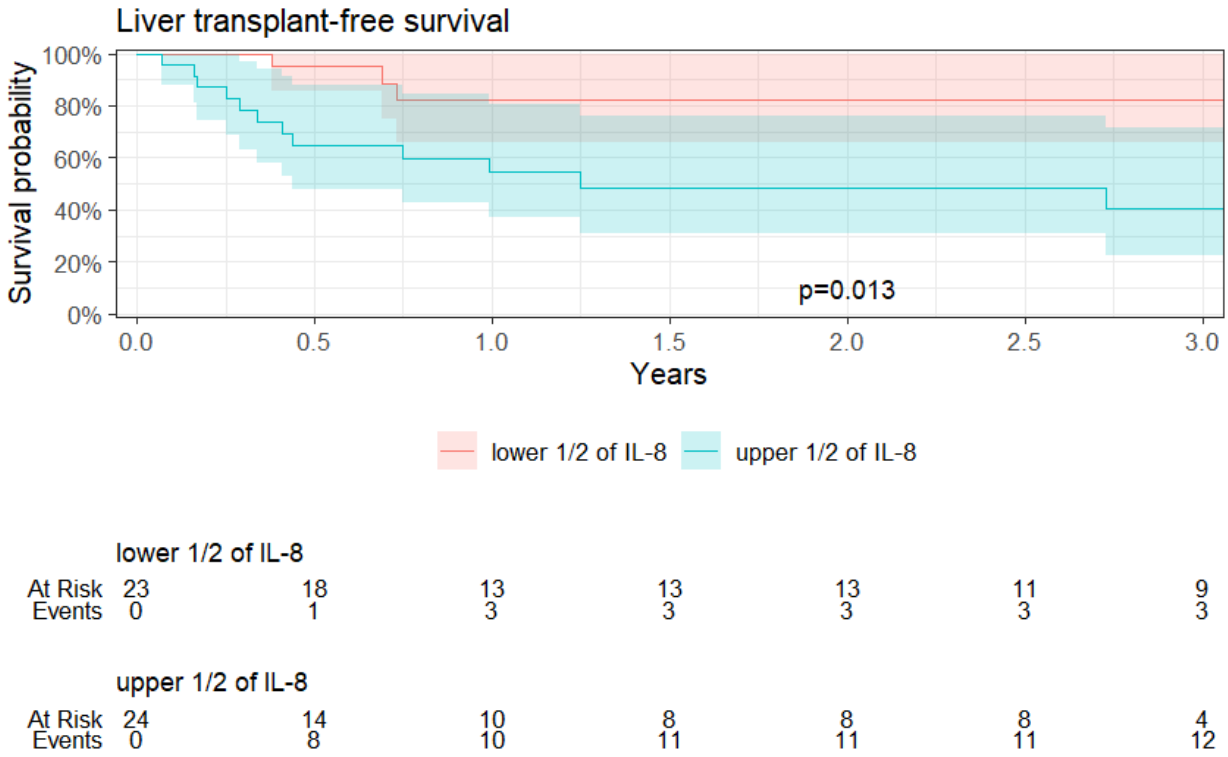
Background and aims: Primary sclerosing cholangitis (PSC) is a chronic fibroinflammatory, progressive liver disease with a high morbidity and mortality burden since no pharmacological treatments are available that can slow down disease progression. Non-invasive biomarkers are required to reliably stratify PSC subgroups and to identify these patients that have a high risk to progress to end-stage liver disease. Immune dysregulation is involved in the pathogenesis of PSC and could serve as a target in the search for new biomarkers. Therefore, we analysed cytokine and chemokine profiles in the sera of PSC patients.

Method: PSC was diagnosed according to clinical criteria. Sera from patients with PSC, without prior liver transplantation, and from healthy controls (HCs, i.e. participants without liver and/or intestinal disease) were prospectively collected and were analysed for a panel of cytokines/chemokines via a multiplex Luminex assay. All samplings occurred during scheduled outpatient visits in a tertiary hospital, in the absence of clinical or biochemical evidence of ongoing active liver and/or gut inflammation. Statistical analyses of independent groups was done with a Mann-Whitney-U test and correlations with continuous variables were made with Spearman's rank correlation coefficient. Kaplan-Meier curves with log-rank tests for liver-transplantation free survival were analysed for each cyto-/chemokine.

Results: we analysed 57 patients with PSC, of which 72% were male, and 15 age- and sex-matched HCs. The mean age at diagnosis (SD) was 35.6 (16.1) years and the median follow-up (IQR) was 6.2 (1.2-12.1) years. 74.5% of patients had large duct disease and 61.4% had concomitant IBD (of which 74% had UC, 20% CD and 6% undetermined IBD). Of all analysed markers, CXCL1 ($p < 0.001$), IL-8 ($p < 0.001$) and sCD163 ($p = 0.001$) showed significantly elevated levels in PSC patients compared with HCs. Surrogate biochemical markers of cholestasis were significantly positively correlated with CXCL1 (Spearman's rho, p value; ALP: 0.40, $p < 0.01$; G-GT: 0.49, $p < 0.001$) and with IL-8 (ALP: 0.51, $p < 0.001$; G-GT: 0.58, $p < 0.001$; total bilirubin: 0.35, $p < 0.05$), while CXCL1 (ALT: 0.47, $p < 0.001$; AST: 0.52, $p < 0.001$), IL-8 (ALT: 0.60, $p < 0.001$; AST: 0.66, $p < 0.001$) and sCD163 (ALT: 0.30, $p = 0.04$; AST: 0.30, $p = 0.04$) were significantly correlated with markers of liver injury. Importantly, elevated IL-8 levels were able to predict liver transplant-free survival (Figure).

Conclusion: CXCL1, IL-8 and sCD163 are reliable biomarkers to stratify patients with PSC from HCs. Moreover, in patients with PSC, serum IL-8 levels reliably identify patients that have a high risk of progression to end-stage liver disease.

Figure:


Liver transplant-free survival
Survival probability
100%
80%
60%
40%
20%
0%
p=0.013
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Years
lower 1/2 of IL-8
upper 1/2 of IL-8
lower 1/2 of IL-8
At Risk 23 18 13 13 13 11 9
Events 0 1 3 3 3 3 3
upper 1/2 of IL-8
At Risk 24 14 10 8 8 8 4
Events 0 8 10 11 11 11 12

P01-09-YI
Beta 7 integrin and L-selectin contribute to cholestatic liver disease in mice

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Background and aims: Directed migration of immune cells contributes to homeostasis and inflammation in the gut-liver unit and is guided by chemokines and adhesion molecules (AMs). The endothelial expressed mucosal addressin cell-adhesion molecule-1 (MAdCAM-1) and its binding partners beta 7 integrin and L-selectin participate in the pathogenesis of gastrointestinal diseases including inflammatory bowel disease (IBD), non-alcoholic steatohepatitis (NASH), and immune mediated hepatitis. Primary sclerosing cholangitis (PSC) is a long-term progressive disease of the intra- and extrahepatic bile ducts, frequently developing as extraintestinal manifestation of IBD. Livers of PSC patients show upregulated MAdCAM-1 expression on hepatic sinusoidal endothelium suggesting for a contribution of misdirected immune cell migration-guided by β7 integrin or L-selectin-to the inflammatory process. Our objective was to study the role of β7 integrin and L-selectin expressing cells and their migration behavior in the context of a chemically induced cholangiopathy mouse model, which resembles aspects of human PSC.

Method: Wildtype mice (WT), L-selectin-deficient and beta7 integrin deficient mice on the C57BL/6J background were fed with a diet supplemented with 0, 1% of 3, 5-diethoxycarbonyl-1, 4-dihydrocollidine (DDC) for four weeks. In addition, $CD8^+$ T cells from WT or beta 7 integrin-deficient mice were adoptively transferred into beta 7 integrin-deficient mice shortly before a four-week DDC feeding. The progression of cholangiopathy in these mice was evaluated by serum biochemistry, liver histology and flow cytometry.

Results: AM-deficient mice showed decreased liver damage, reflected in lower serum levels of bilirubin and alkaline phosphatase, compared to WT mice. Sirius Red staining revealed reduced fibrosis in these mice. Moreover, more hepatic $CD8^+$ T cells could be seen in AM-deficient mice when compared to similarly treated WT mice. In addition, transfer of WT $CD8^+$ T cells caused more severe liver damage in DDC-treated beta 7 integrin-deficient mice than transfer of beta 7 integrin-deficient $CD8^+$ T cells.

Conclusion: In conclusion we have shown that beta 7 integrin and L-selectin contribute to DDC-induced cholangiopathy, most probably by guiding hepatic $CD8^+$ T cell migration. Thus, our study contributes to better understanding of the cell migration pathways associated with the pathogenesis of experimental cholangiopathy and identifies $CD8^+$ T cells as a potential target for specific antiadhesive drugs.

P02-01-YI
Liquid biopsy protein biomarkers of cholangiocarcinoma risk, early diagnosis and survival mirroring tumor cells

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Background and aims: Cholangiocarcinoma (CCA), heterogeneous biliary tumors with dismal prognosis, lacks accurate early diagnostic methods, especially important for individuals at high-risk (i.e., primary sclerosing cholangitis (PSC)). Here, we searched for protein biomarkers in serum extracellular vesicles (EVs).

Method: EVs from patients with isolated PSC (n = 45), concomitant PSC-CCA (n = 44), PSC who developed CCA during follow-up (PSC to CCA; n = 25), CCAs from non-PSC etiology (n = 56), hepatocellular carcinoma (HCC; n = 34) and healthy individuals (n = 56) were characterized by mass-spectrometry. Diagnostic biomarkers for PSC-CCA, non-PSC CCA or CCAs regardless etiology (Pan-CCAs) were defined and validated by ELISA. Their expression was evaluated in CCA tumors at single-cell level. Prognostic EV-biomarkers for CCA were investigated.

Results: High-throughput proteomics of EVs identified diagnostic biomarkers for PSC-CCA, non-PSC CCA or Pan-CCA, and for the differential diagnosis of intrahepatic CCA and HCC, that were cross-validated by ELISA using total serum. Machine learning-based algorithms disclosed CRP/FIBRINOGEN/FRIL for the diagnosis of PSC-CCA (local disease (LD)) vs isolated PSC (AUC = 0.947;OR = 36.9), and combined with CA19-9, overpowers CA19-9 alone. CRP/PIGR/VWF allowed the diagnosis of LD non-PSC CCAs vs healthy individuals (AUC = 0.992;OR = 387.5). Noteworthy, CRP/FRIL accurately diagnosed LD Pan-CCA (AUC = 0.941;OR = 89.4). Levels of CRP/FIBRINOGEN/FRIL/PIGR showed predictive capacity for CCA development in PSC before clinical evidences of malignancy. Multi-organ transcriptomic analysis revealed that serum EV-biomarkers were mostly expressed in hepatobiliary tissues, and scRNA-seq and immunofluorescence analysis of CCA tumors showed their presence mainly in malignant cholangiocytes. Multivariable analysis unveiled EV-prognostic biomarkers, with COMP/GNAI2/CFAI and ACTN1/MYCT1/PF4V associated negatively or positively to patients' survival, respectively.

Conclusion: Serum EVs contain protein biomarkers for the prediction, early diagnosis and prognosis estimation of CCA detectable using total serum, representing a tumor cell-derived liquid biopsy tool for personalized medicine.

P02-02
Functional dichotomy of biliary primary cilia in liver injury and cholangiocarcinoma development

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Background and aims: We have previously published that Primary cilia (Pc) mediate canonical Hedgehog (Hh) signalling in the ductular reaction (DR) in experimental and human cirrhosis. However, our recent publication indicated that Pc loss in biliary epithelial cells (BEC) induced the proliferation of BECs and biliary cystic formation in experimental fibrosis models. We now aim to examine the effect of Pc loss on the development of cholangiocarcinoma in BEC-specific PTEN knockout (KO) mice.

Method: C57BL/6J-congenic Ptenflox and kif3aflox mice crossed with CK19CreERT knock-in mice to produce BEC-specific KO of PTEN, kif3a (Pc loss) or both (DKO) following tamoxifen (TAM) administration in adult mice. Thioacetamide (TAA) was used to induce liver injury and fibrosis for up to 20 weeks. Mice were culled at weeks 14 and 18 post-TAM administration. Tissue samples were examined with histopathological assays and RNA-seq. The SLHD Animal Welfare Committee approved animal breeding and experimental protocols.

Results: In BEC-specific PTEN KO mice (PTEN-BKO), intraductal papillary neoplasm of the bile duct was evidenced in the large intrahepatic bile ducts. TAA administration induced cholangiocarcinoma (CCA) formation. Pc were lost in these CCA cells. To test whether Pc loss actually played a role in the development of CCA, PTEN-BKO mice were crossed with BEC-specific kif3a KO mice to produce double KO (DKO) mice. When these DKO mice consumbed TAA, the number and size of CCAs were significantly increased compared with PTEN-BKO mice. Histological analysis indicated increased expression of PCNA, phospho-FRS2, Sox9, phospho-ERK, infiltration of neutrophils and macrophages, and the presence of significant stroma formation similar to that seen in human CCA. RNA-seq analysis revealed that expression levels of 288 genes were significantly increased in PTEN-BKO and DKO liver tissues compared with wild-type (WT) liver tissues. For example, CCA tumour marker genes, such as msln, CA4, CA9, S100A6, and DMBT1, were dramatically increased in PTEN-BKO and DKO livers compared with WT livers. More importantly, a set of genes over-expressed in human CCA with poor prognosis were enriched in PTEN-BKO and DKO liver tissues. Multiple MAPK genes or signalling pathways and some stem cell markers, such as CD44 and DCLK1, were upregulated in PTEN KO or DKO livers. Interestingly, more than 8% of up-expressed genes in PTEN-BKO and DKO livers are targets of the JNK pathway, which was well-documented to promote CCA growth. Further analysis is ongoing.

Conclusion: Loss of Pc in BEC could have been secondary to the oncogenic transformation, but the increase of CCA in DKO mice indicates a role for Pc in the progression of CCA. Cholangiocarcinoma in such mice shared significant features of human CCA, including many overlapping upregulated genes, thus providing a pre-clinical model for testing new therapeutic approaches.

P02-03-YI
Hematopoietic-biliary organoids as a model for liver development and injury

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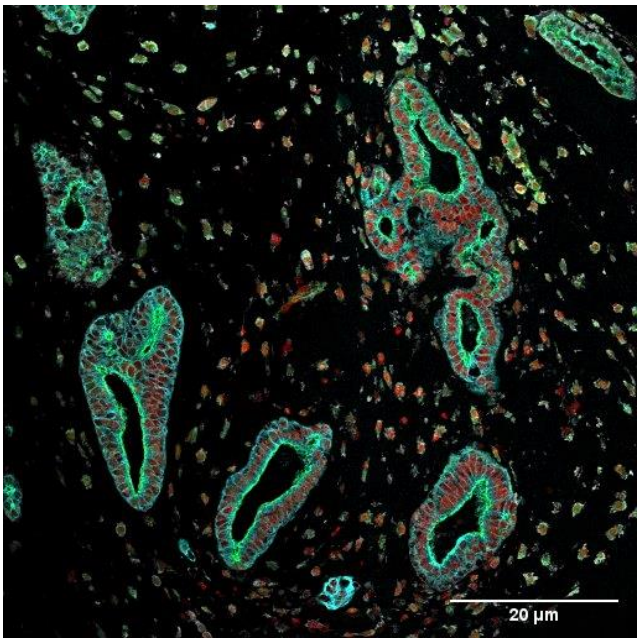
Background and aims: In mammalians, fetal liver hematopoiesis occurs in close proximity to the developing bile ducts. A better understanding of this co-development in humans is necessary for understanding infant cholestatic diseases such as biliary atresia. We herein present our preliminary findings on generating hematopoietic-biliary organoids from human induced pluripotent stem cells (hiPSCs), as a model to study development and response to early injuries of the liver.

Method: Human induced pluripotent stem cells were co-differentiated to endoderm-to-hepatoblast and hemogenic yolk sac mesoderm-to-hematopoietic lineages and embedded as clusters into Matrigel. We used Notch agonists (JAG1 or DLL1) to promote biliary differentiation and a hematopoietic cytokine cocktail to induce hematopoietic differentiation. Biliatresone was used to induce biliary epithelial cell injury. For organoid characterization in control and injured conditions, we performed flow cytometry and high-end multiplexed confocal microscopy.

Results: We successfully generated FOXA2+/CXCR4+ endoderm (62% of total cells) and a smaller population of KDR+/CD235a+ yolk sac mesoderm (6%) that gave rise to CD34+/CD45dim hematopoietic progenitors. We detected bipotential CK19+/HepPar1+ hepatoblast-like cells in the presence of JAG1. Interestingly, only after adding hematopoietic cytokines, we observed cells individually expressing CK19 and HepPar1 and luminal morphogenesis indicating organoid maturation. Biliary growth cytokines EGF and HGF further promoted this process. Subsequent analysis revealed alpha-SMA+ fibroblasts and CD31+ endothelial cells surrounding the biliary cells, CD11b+ myeloid cells, and scattered IBA1+ macrophages reminiscent of the spatial composition of the human fetal liver. Biliatresone did not induce morphological changes, but resulted in a greater emergence of biliary ductular-like structures, resembling a ductular reaction observed during liver pathogenesis.

Conclusion: In summary, our organoid system recapitulates key elements of the fetal liver environment, enabling hematopoietic-hepatobiliary co-development. Our aims are now to improve further this system for elaborating a clinically relevant model of neonatal autoinflammatory cholangiopathies, such as biliary atresia.

Figure: High-end multiplexed confocal microscopy of organoids showing luminal biliary cells surrounded by other cell populations. Markers are CK19 (cyan), ZO-1 (green), alpha-Tubulin (red) and DAPI (grey).



P02-04
Identification of the role of osteopontin in biliary atresia

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Background and aims: biliary atresia (BA) is a devastating neonatal cholangiopathy that leads to cholestasis and progressive hepatic failure. The poor understanding of the pathogenesis and progression of BA results in compromised transplant-free survival. Osteopontin (OPN), encoded by the *SPP1* gene, is highly expressed in biliary epithelial cells (BECs) and is involved in chronic liver disease; however, whether OPN participates in BA is unknown.

Method: the *SPP1* gene expression profile in BA patients was analyzed using publicly available datasets of scRNA-seq, RNA-seq and microarrays. OPN protein expression in BA livers was analyzed by immunohistochemistry. To induce BA in mice, we inoculated i.p. rhesus *rotavirus* (*RRV*) to BALB/c pups within 24 hours of birth, which resulted in BEC infection and bile duct obstruction. Control mice were injected with saline solution. Symptoms of BA were monitored after RRV inoculation and mice were sacrificed at day 11. The activity of transaminases and the levels of total bilirubin were measured in serum, histopathological changes were assessed and OPN levels in serum, urine and liver were determined.

Results: in humans, *SPP1* is predominantly expressed in BECs in healthy individuals and is highly increased in cirrhosis. Notably, *SPP1* gene expression is significantly higher in livers from BA patients compared to non-BA cholestatic liver disease or control pediatric liver donors (GSE 122340, GSE46960). *SPP1* gene expression highly correlates with *KRT7*, *KRT19* and *MMP7*, all markers of BECs. BA patients with fibrosis exhibit higher *SPP1* expression than those with inflammation only (GSE 15235). Mice with BA show delayed development, jaundice and acholic stools. Moreover, they have increased activity of transaminases and levels of total bilirubin. The HandE staining reveals massive ductular reaction and hepatic inflammation. The concentration of OPN in serum and urine and the hepatic expression of OPN are significantly increased in mice with BA compared to controls.

Conclusion: OPN expression is significantly increased in patients and mice with BA and correlates with BEC markers. To dissect if OPN participates in the pathogenesis and progression of BA, our laboratory has generated a mouse model with ablation or overexpression of *Spp1* in BECs. These mice will be inoculated with RRV to elucidate the role of BEC-derived OPN in BA progression.

P02-06-YI

CD8+ T cells from patient with primary biliary cholangitis express e-cadherin upon T cell receptor activation and invade biliary epithelial cells through e-cadherin-beta-catenin interaction

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Background and aims: The pathogenesis of primary biliary cholangitis (PBC) remains elusive with several unanswered questions. The final destruction of the biliary epithelium is operated by autoreactive CD8+ T cells, yet these cells remain poorly characterised and the mechanism of damage remains to be clarified. CD69+ CD103+ CD8+ T cells have been reported to be key players in PBC pathogenesis. Herein, we further characterised their phenotype, reporting a subset of activated CD8+ T cells expressing the epithelial marker E-cadherin, which allows them to invade biliary epithelium anchoring β-catenin on biliary epithelial cells.

Method: Immunofluorescence staining of human PBC tissues was used to identify and characterise CD8+ T cells infiltrating biliary epithelial cells (BEC). Primary CD8+ and CD4+ T cells from peripheral blood of PBC patients (n = 7) and healthy controls phenotype (n = 7) were assessed pre and post-activation upon antiCD3/28 stimulation. We used *in vitro* high-content imaging platforms to characterise the interaction of activated T cells with human primary BECs. Phenotypic assessment of cell-cell interactions observed within co-cultures between T cells and epithelial cells was performed using immunocytochemistry and confocal microscopy.

Results CD8+ T cells upon TCR stimulation upregulated CD103 and CD69, alongside markers of biliary homing, such as CXCR3, CXCR6 and CD49a. Activated CD8+ T cells from PBC patients expressed the epithelial marker E-cadherin significantly more than control samples (median 22.9, IQR 12.4-37.9 vs median 6.4 IQR 5.9-16; p value = 0.007). This expression was not observed persistently in CD4+ T cells from the same patient (median 14.2 IQR 5.9-24.8 vs median 1.8 IQR 0.4-2.8; p value = 0.001). Furthermore, E-cadherin+ CD8+ T cells express more Granzyme B compared with E-cadherin negative cells (median 55.0 IQR 42.9-61.4 vs median 96 IQR 87.5-98.2; p value = <0.0001). Concurrently, activated CD8+ T cells significantly increased their ability to adhere to and invade BECs *in vitro*. These processes were shown to be mediated by adherens junction-like interactions between E-cadherin on CD8+ T cells and β-catenin on the BEC *in-vitro*. The same interactions were documented in PBC liver tissues *in vivo* using immunofluorescence staining.

Conclusion: TCR stimulation induced E-cadherin expression in CD8+ T cells of PBC patients. These cells had a cytotoxic phenotype and the expression of E-cadherin was instrumental for the invasion of the biliary epithelium. These findings unveil a new player in the PBC immune landscape and a novel mechanism of lymphocyte-BEC interaction.

P02-07-YI
Mitochondrial dynamics and autophagy are disturbed in experimental models of primary biliary cholangitis, contributing to disease pathogenesis

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Background and aims: Primary biliary cholangitis (PBC) is a chronic cholestatic and immune-mediated liver disease of unknown origin. We have previously demonstrated that the expression of microRNA-506 (miR-506) is increased in PBC cholangiocytes, targeting the $Cl^-/HCO3^-$ exchanger AE2 and leading to intracellular pH (pHi) disturbances and PBC-like features, including PDC-E2 overexpression and immune activation. Autophagy, and specifically mitophagy, is a critical cell-intrinsic, quality-control and anti-inflammatory mechanism necessary to eliminate dysfunctional organelles, such as mitochondria. These processes are critically dependent on pHi, but their role in PBC remains unknown. Our aim was to characterize the mitochondrial dynamics and auto (mito)phagy processes in PBC and evaluate their contribution to disease pathogenesis

Method: Experimental *in vitro* PBC models [miR-506-overexpressing cholangiocytes (H69 miR-506) and control cells (H69 miR- and H69)] were characterized by high-throughput proteomics. Moreover, cells were characterized in terms of mitochondrial dynamics, integrity and activity, autophagy/mitophagy and cell viability, in baseline conditions and under the presence of different anti-cholestatic drugs.

Results: High-throughput proteomics revealed that several proteins involved in mitochondrial dynamics and autophagy/mitophagy are altered in H69 miR-506 cholangiocytes. Of note, genes involved in mitochondrial fusion (related to increased functionality) were found downregulated whereas certain fission-related genes (related to damaged mitochondria) were found upregulated. Accordingly, these findings were accompanied by decreased mitochondrial functionality measured by flow cytometry. While the levels of autophagy mediators increased in H69 miR-506 cholangiocytes, the levels of PARKIN, a critical mediator of mitophagy, were found markedly decreased, pinpointing a mitophagy impairment. Moreover, toxic bile acid-induced cell death was potentiated by the presence of auto (mito)phagy inhibitors, particularly in miR-506-overexpressing cells. Interestingly, the incubation of H69 miR-506 cholangiocytes with ursodeoxycholic acid (UDCA), bezafibrate (BZF) or obeticholic acid (OCA) not only normalized PDC-E2 overexpression and energetic metabolism, but also tended to increase mitophagy.

Conclusion: Our data suggest that PBC cholangiocytes are characterized by disturbances in mitochondrial integrity and mitophagy, leading to the accumulation of dysfunctional mitochondria and aberrant presentation of mitochondrial antigens. In addition, different anti-cholestatic drugs are able to modulate these mitochondrial disturbances, pointing out that the regulation of mitophagy represents a novel target in PBC.

P02-08
Identification of a functional relationship between MEK5-ERK5 pathway and hypoxia inducible factor 1-alfa

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Background and aims: Cholangiocarcinoma (CCA) is the second most common liver cancer after hepatocellular carcinoma (HCC) and constitutes a heterogeneous group of malignancies that arise from the epithelium of the biliary tree. Each subtype of CCA has a distinct genetic profile that is reflected in a different pathogenesis and prognosis in patients. Genetic heterogeneity opens many opportunities for new personalized and targeted therapies. Currently, in fact, many selective inhibitors are under study. Recently, our group reported the importance of the Mitogen-activated protein kinase Extracellular signal regulated kinase 5 (ERK5) in supporting the survival and proliferation of CCA cells both in vitro and in vivo. In the attempt to identify additional Achilles heel to be target in CCA, we investigated on the existence of a functional relationship between ERK5 and Hypoxia Inducible Factor -1α (HIF-1α), the main regulator of the response to hypoxia, a condition that is typical of the tumour microenvironment.

Method: The two CCA cell line (CCLP-1 and HUCCT-1) were grown at different time points under normoxia and hypoxia conditions. The protein expression analysis was performed by Western Blot.
For the pharmacological treatments, HIF-1α (KC7F2) and ERK5 (XMD8-92 and JWG-071) inhibitor effects were valued using 3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Results: By Western Blot experiments we found that ERK5 phosphorylation and HIF-1α expression are increased in hypoxia. The increased activity of the latter in hypoxia was confirmed by the consistent increase of its target genes, Carbonic Anhydrase 9 (CAIX) and Glucose transporter 1 (GLUT1). At this point, we performed a combined treatment of ERK5 and HIF-1α inhibitors in vitro and found a greater effect than the single treatments in hypoxic conditions. All together, these results lead to the idea to use this co-therapy to treat CCA, given the low oxygen concentration in tumor environment.

Conclusion: These findings led to the identification of a possible functional relationship between ERK5 and HIF-1α in the regulation of CCA homeostasis and put light on a new possible therapeutic option for CCA patients.

P02-09-YI
Primary cilia as a targetable node between biliary injury, senescence and regeneration in liver transplantation

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Background and aims: Biliary complications are a major cause of morbidity and mortality in liver transplantation, developing in up to 25% of liver transplanted recipients, frequently requiring additional surgical procedures, re-transplantation or, in the absence of a suitable regraft, death. Whilst machine perfusion technology is reducing the incidence of biliary complications, there is still an unmet clinical need for the prevention of biliary strictures. Here, we investigate the role of the primary cilium, a highly-specialised sensory organelle, in biliary injury leading to post-transplant biliary complications.

Method: Human biopsies were used to study the structure and function of primary cilia in liver transplant recipients that develop biliary complications (*N* = 7) in comparison with recipients without biliary complications (*N* = 12). To study the biological effects of the primary cilia during transplantation, we generated murine models that recapitulate liver procurement and cold storage, as well as the *K19CreER^{T} Kif3a$^{flox/flox}$* mouse model to conditionally eliminate the primary cilia in biliary epithelial cells. To explore the molecular mechanisms responsible for the observed phenotypes we used *in vitro* models of ischemia, cellular senescence and primary cilia ablation. Finally, we used pharmacological and genetic approaches to target cellular senescence and the primary cilia, both in mouse models and discarded human donor livers.

Results: Prolonged ischemic periods before transplantation result in ciliary shortening and cellular senescence, an irreversible cell cycle arrest that blocks regeneration. Our results indicate that primary cilia damage results in biliary injury and a loss of regenerative potential. We found that the initiation of senescence negatively impacts primary cilia structure, establishing a negative feedback loop that further impairs regeneration. Finally, we explore how targeted interventions for cellular senescence and/or the stabilisation of the primary cilia, improve biliary regeneration following ischemic injury.

Conclusion: Primary cilia play an essential role in biliary regeneration and we demonstrate that senolytics and cilia-stabilising treatments provide a potential therapeutic opportunity to reduce the rate of biliary complications and improve clinical outcomes in liver transplantation.

P02-10
Neutrophils in the pathogenesis of primary sclerosing cholangitis

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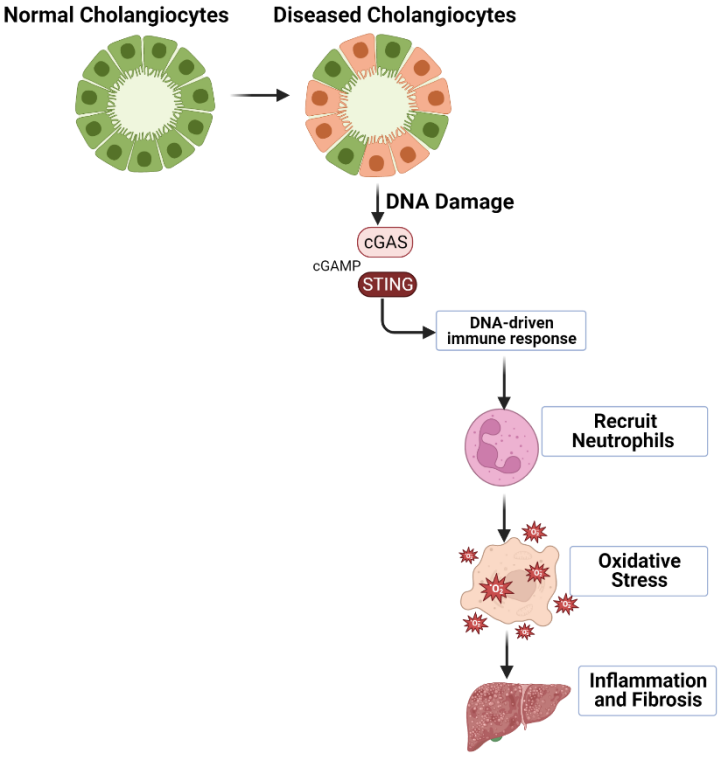
Background and aims: The pathogenesis of Primary Sclerosing Cholangitis (PSC) includes an immune-mediated inflammatory response in which a cascade of pro-inflammatory signals recruits immune cells and perpetuates cholestatic fibrogenesis. The biliary tree is a site of inflammation and immune cell homing; however, bile ducts represent an understudied immunological niche. Neutrophils, the most abundant leukocytes in the blood, infiltrate near the bile ducts of the PSC patients. Nevertheless, the mechanism of neutrophil infiltration in the liver, their association with cholangiocytes, and the crosstalk between neutrophils and cholangiocytes remain obscure.

Method: Immunofluorescence for cholangiocytes and neutrophils was performed on liver tissues from PSC patients and mouse models of PSC (3, 5-Diethoxycarbonyl-1, 4-Dihydrocollidine (DDC)-fed mice and Mdr2-/- mice). Immunohistochemistry was utilized for 8-OHDG (8-hydroxy-2' -deoxyguanosine) to mark oxidative stress in the liver tissue from PSC patients and in mouse models. Biliary organoids from wildtype (WT) and Mdr2-/- mice were utilized for RT-PCR, western blot, and immunofluorescence for DNA damage. RNA sequencing on the organoids was performed followed by pathway analysis of differentially expressed genes (DEG). Liver tissues from mouse models were investigated for activation of the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway.

Results: Mouse models of PSC demonstrate infiltration of neutrophils in close proximity to the bile ducts. RT-PCR shows increased expression of neutrophil markers in liver tissue from Mdr2-/- and DDC-fed mice compared to control chow-fed mice (5-fold and 4.5-fold respectively, n = 5). Western blot reveals increased protein expression of STING in liver tissue from these mice, suggesting activation of the C-GAS STING pathway in these models. Mouse liver tissues from DDC mice as well as biliary organoids from Mdr2-/- mice both demonstrate increased DNA damage in the cholangiocytes by 53-BP1 staining compared to the corresponding WT mouse samples. Ingenuity Pathway analysis on DEGs of WT and Mdr2-/- biliary organoids shows enrichment of mitochondrial dysfunction, DNA damage, and neutrophil extracellular trap signaling. Concurrently, neutrophil infiltration is associated with an increase in oxidative stress in the liver tissue from the mouse models as marked by OHDG staining. Immunofluorescence for STING showed an increased expression around the bile ducts in the DDC mouse liver tissue compared to control.

Conclusion: We propose that DNA damage activates the C-GAS STING pathway to elicit an immune response by recruiting neutrophils in the biliary microenvironment. These events promote oxidative stress and perpetuates the inflammation and biliary fibrosis seen in PSC.

Figure:


Normal Cholangiocytes
Diseased Cholangiocytes
DNA Damage
cGAS
cGAMP
STING
DNA-driven immune response
Recruit Neutrophils
Oxidative Stress
Inflammation and Fibrosis

P03-01
CRISPR-engineered chemically-derived hepatic progenitor cancer organoid recapitulates intrahepatic cholangiocarcinoma carcinogenesis and pathophysiology

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Background and aims: Intrahepatic cholangiocarcinoma (iCC) is a fatal malignancy of the liver's biliary epithelial cells. This cancer has a very low chance of survival, a high degree of heterogeneity, and great difficulty in treatment. A vital discovery that significantly advances cancer research is the development of cancer organoid technique. These organoids can be generated from the specimens of iCC patients, but the effectiveness of this process is very low because of the poor quality and quantity of the sample cells.In addition, cancer organoids can be derived from normal adult stem cells edited by CRISPR technology to emulate the gene mutation that occurred during early carcinogenesis.

Method: To generate the iCC cancer organoid model, we transfected normal human chemically-derived hepatic progenitor cells (hCdHs) with CRISPR-Cas9 plasmid and gRNA plasmids for TP53 and BAP1 gene knock-out by electroporation. Following the transfection, we generated cancer organoid in the matrigel dome from these mutated progenitor cells and performed various analyses on this organoid.

Results: To overcome the current limitation of the patient-derived cancer organoid, we successfully generated cancer organoid from hCdHs that can be massively expanded from a relatively small clinical sample. Then, we introduced the double knock-out mutation of TP53 and BAP1 genes, a well-established iCC cancer driver gene. These CRISPR-engineered hCdHs-derived cancer organoids showed comparable pathophysiological properties with the iCC tumour malignant features. We observed cribriform feature on these organoid, shown by multiple lumen within the organoid, as well as mucous presence in the lumen by PAS and Alcian Blue staining. By immune-staining, we confirmed the pathological features of intrahepatic biliary cell-derived adenocarcinoma ($CK19^{+}$, ALB^{-}) on these organoids and confirmed the presence of mucus including mucin ($MUC1^{+}$) in the organoid lumen.

Conclusion: These results demonstrated the capability of our CRISPR-engineered hCdHs-derived cancer organoid as a powerful intrahepatic cholangiocarcinoma disease modelling platform.

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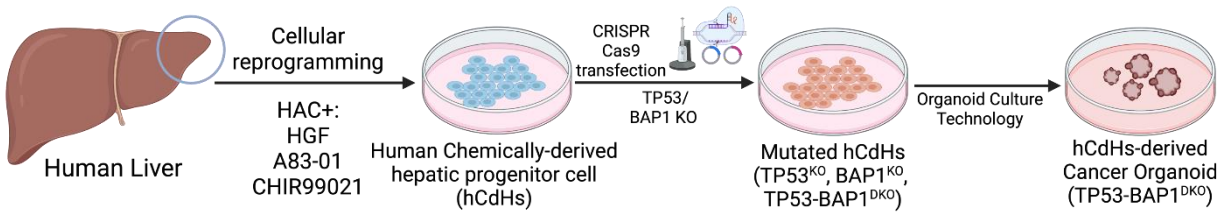
Figure:



P03-02-YI
Metabolic rewiring of cystic cholangiocytes improves mitochondrial dynamics and bioenergetics ameliorating the pathogenesis of polycystic liver disease in experimental models

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Background and aims: Polycystic liver diseases (PLDs) are inherited genetic disorders characterized by progressive development of intrahepatic, fluid-filled biliary cysts, which are main cause of morbidity. Chronic administration of somatostatin analogues, aimed to diminish the increased intracellular cAMP levels is the current pharmacological treatment, but shows modest benefits. The causative genes induce aberrant proteostasis and endoplasmic reticulum (ER) stress in cholangiocytes, promoting cell survival and cystogenesis. Here, we investigated the mitochondrial dynamics, bioenergetics and metabolism, its interaction with the ER, and its therapeutic regulatory value.

Method: Human cystic cholangiocytes (ADPKD$^{GANAB-/-}$ or ADPLD$^{PRKCSH-/-)}$, and normal human cholangiocytes (NHC) as controls, were used. Mitochondrial ultra-structure and its interaction with ER was evaluated by transmission electron microscopy (TEM). Mitochondrial mass, dynamics and functionality were analyzed by flow cytometry and qPCR. Real-time mitochondrial bioenergetic activity was assessed by Seahorse Analyzer, and protein levels of electron transport chain (ETC) complexes by immunoblotting. Metabolic fluxes were analyzed using radiolabelled substrates, and lipid concentration was measured by thin layer chromatography.

Results: Cystic cholangiocytes showed altered mitochondria-associated ER membranes (MAMs), characterized by increased distance between the ER and mitochondria, as well as augmented mitochondrial mass and marked overexpression of mitochondrial dynamics- and biogenesis-related genes compared to NHC. Notably, cystic cholangiocytes were characterized by increased mitochondrial membrane potential, alterations in the mitochondrial bioenergetic profile, including increased [H^+]-leak and maximal respiration rates, as well as, spare respiratory capacity compared to NHC. In accordance, cell ATP content and mitochondrial reactive oxygen species (ROS) levels were increased in cystic cells, which was associated to upregulation of the protein complexes I, III and IV of the ETC. Regarding the energy source, cystic cholangiocytes showed higher lipid β-oxidation rate and triglyceride storage compared to NHC. Interestingly, cystic cells also presented augmented capacity for *de novo* synthesis and storage of free cholesterol. Thus, treatment with statins (Pravastatin or Atorvastatin) halted the mitochondrial bioenergetic hyperactivity and reduced cell proliferation in a dose-dependent manner.

Conclusion: Cystic cholangiocytes show altered mitochondrial bioenergetics and metabolism, leading to increased ATP synthesis, with lipids being an important energy substrate. Rewiring lipid metabolism with statins impact on key cell processes in PLD, such as, ER stress, mitochondrial functionality and cAMP levels, arising as a novel therapeutic opportunity.

P03-03-YI
Large CD103+ CD69+ CD8+ T cells consistently invade biliary epithelial cells in patients with primary biliary cholangitis

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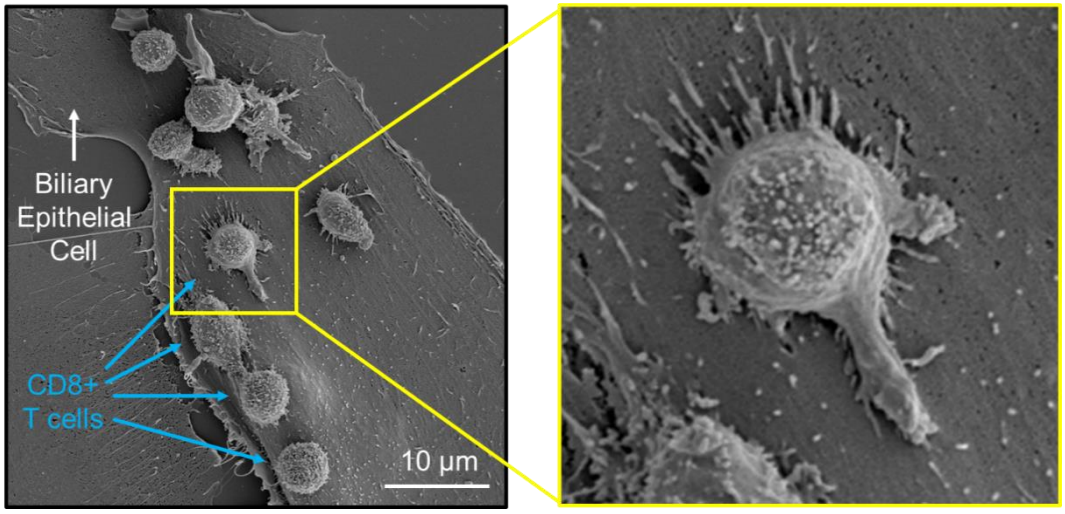
Background and aims: The early immunopathogenic events which precede the development of Primary Biliary Cholangitis (PBC) are unknown. We and others have documented the presence of CD8+ T cells within the cytoplasmic spaces of biliary epithelial cells (BEC). We aimed to uncover the molecular mechanisms of this internalization and its link to PBC pathogenesis.

Method: We developed an *in vitro* high-content imaging (HCI) platform to quantify and characterize the internalization of blood-derived T cells into primary human BEC. This assay was used to observe the effect of T cell receptor activation, to compare CD8+ T cells to donor matched CD4+ T cells (N = 3), and to investigate the effect of small molecular inhibitors (N = 5). Complete internalization was confirmed by confocal and transmission electron microscopy. Ultrastructural assessment of CD8+ T cell internalization was investigated using scanning electron microscopy. T cell phenotyping was performed *in vitro* using flow cytometry and *in vivo* using immunohistochemistry (IHC). We also conducted semi-quantitative analysis using IHC where we documented the localization of CD8+ T cells in the liver which expressed E-cadherin ligands-CD103 or killer-cell lectin like receptor G1 (KLRG1). Here, we compared formalin fixed paraffin embedded (FFPE) liver tissue sections from patients with PBC, autoimmune hepatitis (AIH), alcoholic liver disease (ALD) and non-cirrhotic donors (N = 6/disease).

Results: CD8+ T cell internalization into BEC was more frequent amongst larger cells and required TCR-mediated activation. CD8+ T cells invaded BEC more frequently compared to CD4+ T cells ($p = 0.0167$). CD8+ T cells formed multiple pseudopod-like interactions with the BEC membrane before spreading and eventually invading the epithelial cells (Figure). Inhibitors of Phosphoinositide 3-kinase (PI3K) signaling and actin remodeling (Wortmannin and cytochalasin D, respectively) significantly reduced their internalization into BEC ($p = 0.005$ and 0.030, respectively). CD103 and KLRG1-expressing CD8+ T cells were found adhered to surface of BEC in all diseases. However, internalized CD103+ CD69+ T cells were found in all IHC-stained PBC tissue samples that were analyzed, compared to 0% of AIH, 33% of non-cirrhotic donors and 16% of ALD patients.

Conclusion: CD103+ CD69+ CD8+ T cells invade BEC through processes requiring cell shape changes and actin modelling. This internalization is consistently observed in patients with PBC and is therefore likely to contribute to disease pathogenesis. Further understanding into the development of these T cells and the consequences of this internalization will likely yield therapeutic targets for the treatment of PBC.

Figure:



P03-04-YI
Targeting OX40 signalling to modulate regulatory T cells and reduce biliary fibrosis

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Background and aims: Reduced regulatory T cells (Tregs) numbers, increased bile duct senescence and scarring are observed in primary sclerosing cholangitis (PSC) patients. Cholangiocytes act as facultative liver progenitor cells through ductular reaction during extensive liver damage, whether this process is impaired during PSC remains to be investigated. The role of Tregs in modulating tissue repair has been shown in multiple organs, but this remains unclear in the context of liver regeneration. We aim to use transgenic murine models to investigate the cause and consequences of reduced Tregs and whether Tregs can be modulated through the co-stimulatory pathway OX40 to reduce biliary scarring and enhance biliary regeneration.

Method: Foxp3^{GFP}DTR transgenic mice were used to reduce Tregs number whilst cholestatic liver injury was induced by the feeding of 3, 5-diethoxycarbonyl-1, 4-dyhydrocollidine (DDC) diet and compared to the control group with intact Tregs population. Tregs stability was investigated through using the Foxp3^{GFPCreERT}tdTom^{loxSTOPlox} mice. Tamoxifen was administered to induce tdTomato expression in Foxp3 Tregs before injury, and the fate of the labelled population was investigated after DDC diet. OX40L blocking antibodies were administered to inhibit OX40 signalling during biliary injury. Tregs were isolated from spleen and co-cultured with intrahepatic cholangiocytes organoids to confirm the effect of Tregs on cholangiocytes.

Results: Mice with reduced Tregs have a lower tolerance to the feeding of DDC diet, with rapid weight loss and two times higher periportal fibrosis and myofibroblasts activation. The reduction in Tregs also decreases the magnitude of Ck19^{+} ductular reaction by 30%, with two-fold increase in Ck19+p21+ senescing cholangiocytes when Tregs are reduced during DDC diet. RNA sequencing analysis of isolated bile ducts from mice with reduced Tregs showed downregulation in genesets involved in ductular reaction such as the Yap and Wnt signalling pathways.

The Foxp3 fate mapping experiments showed the labelled Treg population acquire a pro-inflammatory phenotype with increased IFN-γ and *TNF-α* secretion. Inhibiting OX40 signalling during biliary damage increased intrahepatic Tregs numbers and stability, reduced the degree of weight loss induced by the DDC diet. This is also linked with a 30% reduction in periportal fibrosis.

Conclusion: We have shown the roles of Tregs in regulating the landscape of biliary regeneration and fibrosis. The reduction in Tregs causes fibrosis and impairs ductular reaction. Tregs can acquire a pro-inflammatory phenotype in an injured microenvironment which may explain the lack of Tregs seen in PSC patients. Interestingly, the blocking of OX40 signalling increases Tregs in the liver and reduces periportal fibrosis. These show the potential of using Tregs to promote liver regeneration by modulating the OX40 pathway.

P03-05
Combined inhibition of bile salt synthesis and intestinal bile salt uptake ameliorates cholestatic liver damage and reduces colonic bile salt levels in mice

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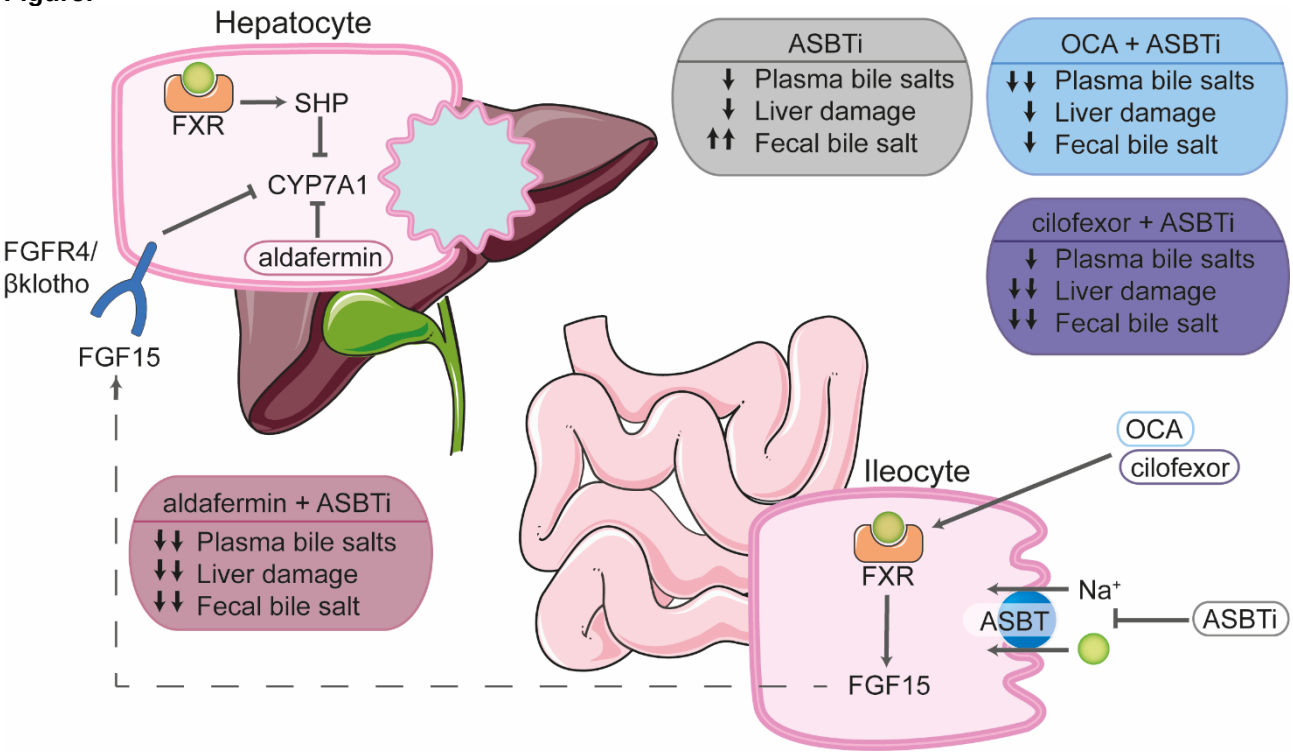
Background and aims: Intestine-restricted inhibitors of the apical sodium-dependent bile acid transporter (ASBT or IBAT) are approved as treatment for several inheritable forms of cholestasis but are also associated with abdominal complaints and diarrhea. Furthermore, blocking ASBT as single therapeutic approach may be less effective in moderate to severe cholestasis. We hypothesized that interventions that lower hepatic bile salt synthesis in addition to intestinal bile salt uptake inhibition provide added therapeutic benefit in the treatment of cholestatic disorders. Here, we test combination therapies of intestinal ASBT inhibition together with obeticholic acid (OCA), cilofexor and the non-tumorigenic Fgf15/FGF19 analogue aldafermin in a mouse model of cholestasis.

Method: Wild type male C57Bl6J/OlaHsd mice were fed a 0.05% 3, 5-diethoxycarbonyl-1, 4-dihydro-collidine (DDC)-diet and received daily oral gavage with either 10mg/kg OCA, 30mg/kg cilofexor, 10mg/kg ASBT inhibitor (Linerixibat; ASBTi) or a combination. Alternatively, wild type male C57Bl6J/OlaHsd mice were injected with AAV8 to express aldafermin, to repress bile salt synthesis, or control AAV8. During a 3 week 0.05% DDC diet, mice received daily oral gavage with 10mg/kg ASBTi or placebo control.

Results: Combination therapy of OCA, cilofexor or aldafermin with ASBTi effectively reduced fecal bile salt excretion. Aldafermin+ASBTi further lowered plasma bile salt levels compared to ASBTi monotherapy. Cilofexor+ASBTi and aldafermin+ASBTi treatment reduced plasma alanine transaminase and aspartate transaminase levels. This suggested improved liver integrity, which was supported by reduced liver cytokeratin 7 and Sirius Red staining. Lastly, inflammatory and fibrotic gene expression was downregulated in cilofexor+ASBTi and aldafermin+ASBTi treated mice.

Conclusion: Combining pharmacological intestinal bile salt uptake inhibition with repression of bile salt synthesis may form an effective treatment strategy to reduce liver injury while dampening the ASBTi-induced colonic bile salt load.

Figure:


Hepatocyte
FXR
SHP
CYP7A1
aldafermin
FGFR4/
βklotho
FGF15
ASBTi
Plasma bile salts
Liver damage
Fecal bile salt
OCA + ASBTi
Plasma bile salts
Liver damage
Fecal bile salt
cilofexor + ASBTi
Plasma bile salts
Liver damage
Fecal bile salt
aldafermin + ASBTi
Plasma bile salts
Liver damage
Fecal bile salt
Ileocyte
OCA
cilofexor
FXR
FGF15
Na+
ASBT
ASBTi

P03-06-YI
Beta-caryophyllene a CB2 specific agonist prevents the development of hepatopulmonary syndrome in experimental model of biliary cirrhosis

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Background and aims: Hepato-Pulmonary syndrome (HPS), a major pulmonary manifestation of liver cirrhosis involves pulmonary arterial vasodilation and impaired oxygen exchange leading to severe hypoxemia and mortality. Cirrhotic milieu contribution in pulmonary remodeling and its mechanisms is largely unknown. Cannabinoid 2 receptor agonism has shown hepatoprotective effects. β-caryophyllene (BCP) a CB2-specific agonist has been reported to reduce hepatic fibrosis in bile duct-ligated rats. Currently, rodent bile duct ligation (BDL) is the only proposed model for HPS, So In this novel study, we investigated the effect of β-caryophyllene on the pulmonary and portal dynamics in the setting of HPS.

Method: In the experimental setup, Sham (Gr1), Bile Duct Ligated (BDL) 4 weeks (Gr2) and BDL+BCP (5mg/kg-oral gavage) (Gr3) were compared hemodynamically. 2-D Echocardiography (cardiac and pulmonary artery changes), Right ventricular systolic pressure (RVSP) and hepatic hemodynamics were monitored. Arterial blood gas (ABG) analysis, histomorphological and molecular mechanisms were investigated.

Results: In BDL animals, portal pressure (mmHg) was increased in Gr2 (15.6 ± 3, +170.5%) vs Gr1 (6.05 ± 0.8); decreased in Gr3 (10.03 ± 0.5, -35.7%) vs Gr2 *P < 0.0001. RVSP (mmHg) was decreased in Gr2 (12.27 ± 3, -43.7%) vs Gr1 (21.8 ± 2); *P < 0.0001; increased in Gr3 (18.66 ± 2, +52%)vs Gr2. M-mode 2D Echo showed that End Diastolic Diameter (EDD) was decreased in both Gr2 and Gr3 vs Gr1. Histo-morphological investigations in lungs revealed pulmonary artery diameter (µm) was increased in Gr2 (60.58 ± 12, +151%), *P < 0.006 in comparison to Gr1 (23.11 ± 3) while decrease in Gr3 (43.2 ± 3, -28%) vs Gr2 *P < 0001 and liver showed significant decrease in fibrosis and ductular reactions in Gr3 as compared to Gr2. ABG showed increased metabolic lactate levels (mmol/l) (cLac) in Gr2 (5.1 ± .2;+410%) vs Gr1 (1 ± .2) and decrease in Gr3 (3.9 ± .5, -23.52%) vs Gr2. Oxygen partial pressure (mmHg) was low in Gr2 (75.9 ± 1;-26.7%) vs Gr1 (103.6 ± 2) and increase in Gr3 (91.23 ± 3;+20.23%); *P < 0.001.Mechanistically, in lungs of Gr2 and Gr3 animals, inflammation, oxidative stress genes and vasodilatory markers were significantly upregulated compared to Gr1 [IL-Beta1 (>6, >4 folds) superoxide dismutase 1 (>7, >4 folds), Klf2 (>5, >2 folds) (*P < 0.05)]. Real-time expression of CB2 was upregulated in Gr2 as compared to Gr1, though Gr3 vs Gr1 was not significant (Fig 1)

Conclusion: Our observation in experimental biliary cirrhosis reveals that BCP administration prevented the development of HPS to a significant extent as compared to the untreated group. Thus, we report for the first time, BCP treatment at an early stage of the disease as a preventive strategy for HPS.

Figure:

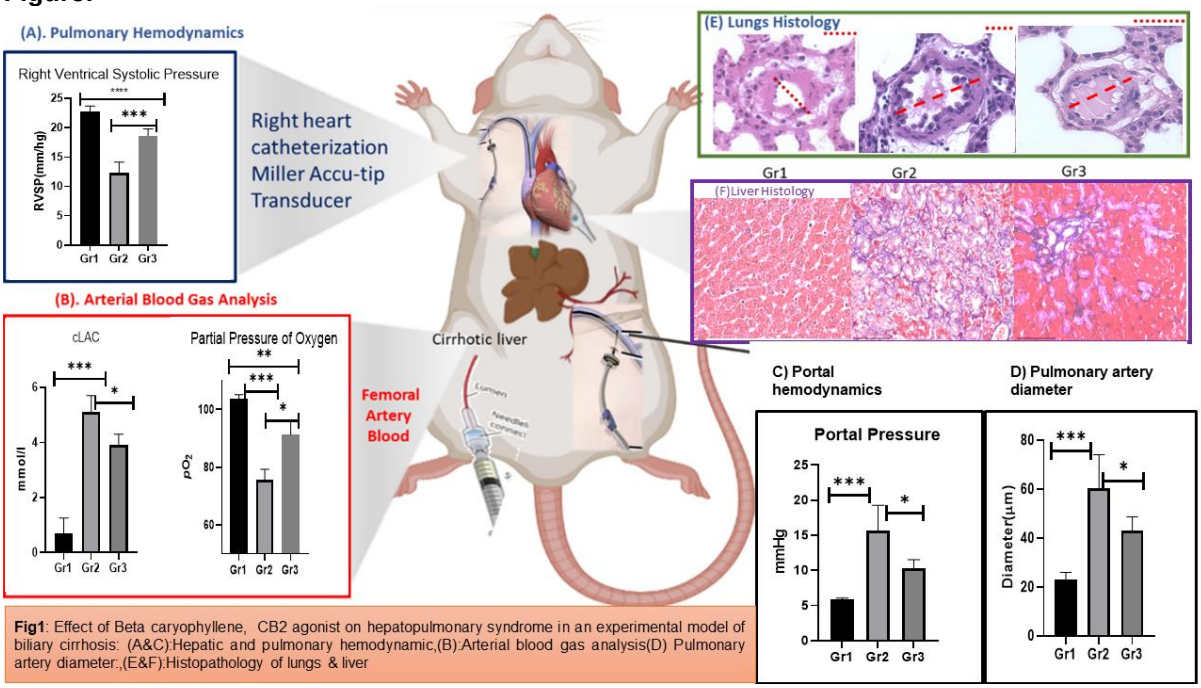

Fig1: Effect of Beta caryophyllene, CB2 agonist on hepatopulmonary syndrome in an experimental model of biliary cirrhosis: (A&C):Hepatic and pulmonary hemodynamic,(B):Arterial blood gas analysis(D) Pulmonary artery diameter:,(E&F):Histopathology of lungs & liver

P03-07
Patient proteomic data and mouse model reinforce the proinflammatory role of CCL24 in cholestatic disease

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Background and aims: CCL24 is a chemokine implicated in inflammation and fibrogenesis. In livers of patients with Primary Sclerosing Cholangitis (PSC), CCL24 and its cognate receptor CCR3 are overexpressed, primarily in the affected biliary area. We previously demonstrated that CCL24 induces monocyte and neutrophil migration, using in vivo and in vitro models. This study investigates the effect of CCL24 on immune cell trafficking in a PSC-related model and explores its concordance with immune-related pathways in patients' serum.

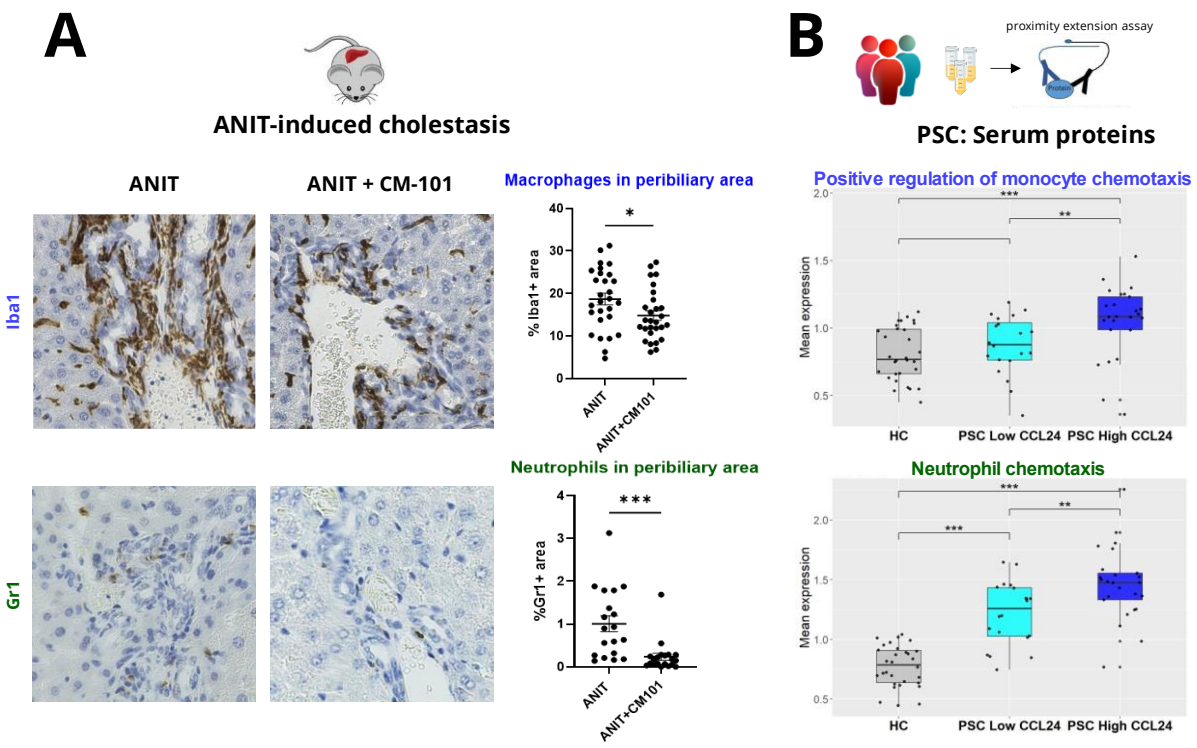
Method: A Chronic α-naphthylisothiocyanate (ANIT)-induced cholestasis mouse model was used to evaluate the effect of CM-101, a monoclonal CCL24 neutralizing antibody that is currently being evaluated in clinical studies, on immune cell trafficking to the liver. Mice were fed with an ANIT diet (0.05%) for 4 weeks and treated twice a week with either 5 mg/kg CM-101 or a vehicle control during weeks 2 through 4. Liver inflammation was evaluated by immunohistochemistry staining for various immune markers. Liver fibrosis and biliary hyperplasia were evaluated by hematoxylin and eosin (HandE) and Pan-CK staining, respectively.

Sera from healthy controls (n = 30) and patients with PSC (n = 45) were evaluated for expression of immune-migration related proteins using the Olink proximity extension assay. Demographics and enhanced liver fibrosis (ELF) scores were captured. The expression of proteins related to pathways associated with immune cell migration was first compared between healthy controls and patients with PSC. Analyses were then further performed among patients with PSC based on serum levels of CCL24 (stratified by the median) and by ELF score (stratified by a score of 9.8).

Results: In the ANIT-induced cholestasis model, CM-101 inhibited the accumulation of peribiliary neutrophils and macrophages. These changes in immune cell populations were correlated with a reduction in disease severity, including amelioration of biliary hyperplasia and fibrosis, following CM-101 treatment.
In patients with PSC, high CCL24 levels were found to be associated with upregulation of monocyte and neutrophil chemotaxis pathways. The same pathways were found to correlate with disease and fibrosis severity.

Conclusion: This study further substantiates the role of CCL24 in inflammation and fibrogenesis. Specifically, it appears to play a role in the recruitment of immune cells and disease severity in patients with PSC. Targeting CCL24 with the investigational drug (CM-101) may be a therapeutic approach for treating patients with PSC as it could inhibit the recruitment of immune cells to the liver and reduce liver inflammation and fibrosis.

Figure:


A
ANIT-induced cholestasis
ANIT
ANIT + CM-101
Iba1
Gr1
Macrophages in peribiliary area
% Iba1+ area
ANIT
ANIT+CM101
Neutrophils in peribiliary area
%Gr1+ area
B
proximity extension assay
PSC: Serum proteins
Positive regulation of monocyte chemotaxis
Mean expression
HC
PSC Low CCL24
PSC High CCL24
Neutrophil chemotaxis

P03-10-YI
A3907, a systemic ASBT inhibitor, improves cholestasis and biliary fibrosis in mice by multi-organ activity and shows translational relevance to human

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Background and aims: Cholestasis is a severe clinical manifestation of cholangiopathies in which impaired bile flow leads to intrahepatic accumulation of bile constituents, including bile acids (BAs). Sustained cholestasis can progress to advanced stages of liver disease and eventually liver failure. The apical sodium-dependent BA transporter (ASBT) drives BA reabsorption in the ileum, bile ducts and kidneys, contributing to BA overload during cholestasis by preventing BA excretion. Our aim was to explore the therapeutic and translational potential of a novel oral systemic ASBT inhibitor (A3907) for the treatment of cholestasis.

Method: The pharmacological profile of A3907, its therapeutic potential in experimental models of cholestasis (i.e., *Mdr2*$^{-/-}$ and BDL mice), and its translational potential in healthy human subjects was investigated.

Results: A3907 was a potent and selective inhibitor of both mouse and human ASBT displaying significant systemic biodistribution in healthy rodents and in experimental models of cholestasis. In wild-type mice, daily oral administration of A3907 for 7 days promoted fecal BA excretion without affecting urine BA levels. In *Mdr2*$^{-/-}$ mice, 4-week oral treatment with A3907 significantly reduced liver- and spleen-to-body weight ratios, serum BAs, plasma markers of liver injury, and liver markers of inflammation, fibrosis, and ductular reaction. RNAseq analysis of livers from the *Mdr2*$^{-/-}$ mice identified biological processes such as fibrosis, inflammation, cell proliferation and apoptosis, endoplasmic reticulum stress and steroid metabolism being beneficially modulated by A3907. Moreover, A3907 prevented apoptosis in cultured cholangiocytes exposed to high concentrations of BA, indicating direct cholangio-protective capacity. In BDL mice, A3907 orally administered for 11 days enhanced renal BA clearance resulting in reduced serum and biliary BA levels. The pronounced body weight loss observed in BDL mice was prevented by A3907 administration. A3907 treatment led to marked reduction in serum AST, ALT, bilirubin, and urea, and reduced inflammatory cell infiltration and necrotic areas in the treated animals. Finally, the translational potential of A3907 was investigated in a placebo-controlled Phase 1 study, showing that A3907 was well-tolerated in human subjects at pharmacologically active doses that led to reduced serum BA and LDL cholesterol.

Conclusion: A3907 is the first oral systemic ASBT inhibitor that acts at the level of the intestine, liver and kidney and robustly attenuates cholestatic liver damage in experimental models. A3907 was well-tolerated in human subjects at doses reaching systemic exposures comparable to those required for therapeutic effects in animal models of cholestasis. Collectively these results highlight the promising translational potential of A3907 for the treatment of cholestatic diseases.

P04-02-YI
Mice lacking the NO receptor develop biliary fibrosis and become worsen upon feeding Western diet

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Background and aims: Biliary fibrosis, resulting from primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), is one of the leading causes of cirrhosis. Early optimism for a robust antifibrotic agent has so for proved premature both in biliary fibrosis and in liver fibrosis in general mainly due to the lack of full understanding of mechanistic drivers. In the present study, we have characterized the role of the NO receptor, NO-sensitive guanylyl cyclase, in biliary fibrosis.

Method: 8-10-week-old male mice carrying a global deletion of NO-GC and their wildtype siblings (C57BL/6J background) were fed control diet and Western diet (21 % fat, 0.2 % cholesterol and 42 g/l fructose) for 16 weeks. Thereafter, mice were sacrificed, and liver tissues were fixed in paraformaldehyde for histological evaluation to assess the rate of disease progression. Analyses included HE and Sirius red staining.

Results: NO-GC knock mice on control diet had altered ductular architecture of the biliary system and increased ductular proliferation (between stage 1 and stage 2) which worsened on Western diet (≥stage 2). In contrast, wildtype siblings were normal after the 16-week period. Moreover, NO-GC KO mice on control diet had periportal inflammation and mild expansion of portal tracs. The severity of the periportal inflammation and the subsequent expansion of the portal tracs were higher in NO-GC KO mice than in wildtype siblings upon feeding Western diet. Moreover, NO-GC KO knockout mice had mild septal fibrosis with a classical periductal onion skin fibrosis which increased upon feeding Western diet. Surprisingly, NAFLD/NASH related classical events (steatosis, steatohepatitis and chicken wire fibrosis) were completely absent in NO-GC KO mice on Western diet whereas wildtype siblings did show the NAFLD/NASH features.

Conclusion: Deletion of NO-GC promotes ductular proliferation and biliary fibrosis. These symptoms worsen upon feeding Western diet. Thus, we postulate a so far unknown role of NO-GC in preventing biliary fibrosis.

P04-03
Thy-1 expression in liver histology correlates with fibrosis grading in patients with primary biliary cholangitis

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Background and aims: Portal myofibroblasts (PMF) have been shown to be important in bile duct injury, but their relevance in liver fibrogenesis is less understood than the established role of hepatic stellate cells (HSC). Thy-1 is an adhesion molecule that controls many core functions of fibroblasts relevant to fibrogenesis and is also present in serum in a soluble form (sThy-1). Thy-1 is expressed *in vitro* by PMF but not HSC. *In vivo* data in humans and data on early and late fibrosis are lacking. Our aim was to investigate whether Thy-1 expression in liver histology and serum sThy-1 levels correlate with the degree of liver fibrosis in patients with primary biliary cholangitis (PBC).

Method: Liver histologies were stained with a Thy-1 antibody, an anti-alpha-smooth muscle actin antibody (SMA) as a fibroblast control, and Masson's trichome for fibrosis assessment. The analysis was semi-quantitative with four categories of staining intensity (weak, low, medium, and strong). METAVIR score was used to describe the degree of fibrosis and three disease groups were defined (F0/1, F2, and F3/4). sThy-1 was measured using an enzyme-linked immunosorbent assay (ELISA). Association was assessed using ordinal logistic regression expressed as odds ratio (OR) and 95% confidence interval (CI).

Results: Samples from 68 patients with PBC were included. The Median (IQR) age at histology was 59 years (46-58.5), with 82.3% being women. METAVIR F0/F1 was present in 30.9% of patients on histology, F2 in 32.35%, and F3/4 in 36.7%. Thy-1 expression was weak in 5.9%, low in 54.4%, medium in 27.9%, and strong in 11.8%. Thy-1 staining intensity correlated significantly with fibrosis severity (OR 10.8, CI 4.07-28.7, p <0.001). The correlation with fibrosis was also significant for the intensity of *periportal* SMA (OR 4.98, CI 2.4-10.3, p <0.001) and *sinusoidal* SMA intensity (OR 2.1, CI 1.1-3.8, p 0.02). In all patients (94.1%) but the ones with only weak Thy-1 expression, Thy-1 and SMA were colocalized *periportal* (p <0.001). In comparison, *sinusoidal* colocalization of Thy-1 and SMA was present in 21.6% of patients with low Thy-1 expression, 68.4% of medium Thy-1 expression, and 100% of strong Thy-1 expression (OR 17.2, CI 5.3-56, p <0.001).

Preliminary sThy-1 measurements in five of the patients with PBC-related liver cirrhosis was significantly elevated in comparison to five healthy controls with comparable age and sex distribution (p 0.026).

Conclusion: Thy-1 expression in liver histology in patients with PBC correlates significantly with the degree of fibrosis. The expression pattern from only periportal in mild fibrosis to sinusoidal in advanced fibrosis might represent the expansion of PMF from periportal to sinusoidal as the disease progresses. Our next steps are completion of sThy-1 measurements in our PBC patients and comparison with patients with metabolic-associated liver disease.

P04-04
Primary biliary cholangitis symptoms are reduced in Mcpip1fl/flAlbCre mice after pharmacological treatment

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Background and aims: Primary biliary cholangitis (PBC) is a chronic autoimmune liver disease that results from slow, progressive destruction of the intrahepatic bile ducts. Mcpip1$^{fl/fl}$Alb^{Cre} mice depleted of Mcpip1 protein in the liver epithelial cells develop a number of typical PBC symptoms. They are characterized by high total bile acids level and increased concentrations of antimitochondrial and antinuclear antibodies in the serum. Mcpip1$^{fl/fl}$Alb^{Cre} animals are also characterised by bile duct pathology which includes hyperplasia of the intrahepatic bile ducts, bile duct epithelium disruption, fibrosis and liver inflammation in comparison to Mcpip1$^{fl/fl}$ controls. The aim of this project is to analyse PBC symptoms in those mice after oral administration of first- and second-line therapy used in patients suffering from PBC or a Lakcid probiotic (*Lactobacillus rhamnosus*).

Method: Mcpip1$^{fl/fl}$Alb^{Cre} knockout and Mcpip1$^{fl/fl}$ control male mice were used. At the age of 6 weeks, mice were randomly divided into five groups for daily oral drug treatment: 1) control (corn oil); 2) Lakcid (*L. rhamnosus*, 10^9 CFU/day per mouse); 3) ursodeoxycholic acid (UDCA, 15 mg/kg body weight per mouse daily); 4) UDCA and Lakcid; 5) UDCA and obeticholic acid (OCA, 10 mg/kg body weight per mouse daily). After 6 weeks of treatment all mice were sacrificed, and the collected material was analyzed by biochemical and ELISA serum tests, histological stainings (hematoxylin and eosin, picro Sirius red) and qPCRs.

Results: Control Mcpip1$^{fl/fl}$Alb^{Cre} knockout mice after 6 weeks of treatment with corn oil were characterized by high serum levels of total IgM, total bile acids and anti-PDC-E2 autoantibodies in comparison to Mcpip1$^{fl/fl}$ counterparts. Significant fibrosis and proliferation of cholangiocytes were also detected in those mice, together with high expression of *Tgfb1*, *Il1b*, *Ngp* and *Ngf*. Out of Mcpip1$^{fl/fl}$Alb^{Cre} treated with different drugs, mice that received Lakcid responded in the best way. Administration of Lakcid led to the reduction of total bile acids in the serum and decreased proliferation of cholangiocytes. Additionally, Mcpip1$^{fl/fl}$Alb^{Cre} mice gavaged with Lakcid had reduced amount of PDC-E2 autoantibodies to the level observed in Mcpip1$^{fl/fl}$ counterparts. Mice from this group were chosen for further multiomic analysis of livers (next generation sequencing, mass spectrometry) that are currently ongoing. Treatment with UDCA or with UDCA+OCA also reduced serum levels of TBA in Mcpip1$^{fl/fl}$Alb^{Cre} mice, but had no effect on cholangioctes' proliferation nor gene expression in the livers.

Conclusion: Treatment of Mcpip1$^{fl/fl}$Alb^{Cre} mice with Lakcid had beneficial effects on PBC symptoms. We hope that analysis of Mcpip1$^{fl/fl}$Alb^{Cre} may shed new light on the pathology of PBC development.

P04-05-YI
Mertk-expressing macrophages promote the malignant features of cholangiocarcinoma cells

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Background and aims: A typical feature of cholangiocarcinoma (CCA) is a dense stromal reaction populated by fibrogenic myofibroblasts and immune cells. The myeloid-epithelial-reproductive tyrosine kinase (MerTK) is highly expressed by a macrophage subset defined as M2c. We investigated whether signals generated by MerTK-expressing macrophages (MØs) modulate the biology of CCA.

Method: 3D-tumor sphere (SPH) cultures enriched in cancer stem cells were generated from intrahepatic CCA (iCCA) cell lines (HuCCT-1 and CCLP-1). Circulating monocytes were differentiated into M2c MØs *in vitro*. Recombinant Gas-6, a MerTK ligand, was used to activate MerTK. *MERTK* expression in human CCA tissues was analyzed at mRNA level in public database and confirmed by immunohistochemistry (IMH) (n = 74). Single-cell RNA sequencing of $CD45^+$ sorted cells was performed in paired non-tumoral and tumoral specimens from intrahepatic CCA patients (n = 6).

Results: Conditioned media (CM) of iCCA SPH induced higher MerTK expression in monocytes compared to CM obtained from cells cultured in monolayers. Conversely, soluble mediators released by Gas6-stimulated M2c MØs, which express MerTK at high levels, increased sphere number and volume and drug-resistance in iCCA cells. These effects were reduced by UNC2025, a small molecule inhibitor of MerTK. Similarly, CM from Gas6-stimulated M2c MØs increased the invasive capacities and expression of stem-like genes of iCCA cells in a co-culture system. Transcriptomic analysis of laser-captured, micro-dissected epithelium and stroma from 23 iCCA patients showed that *MerTK* mRNA expression is significantly higher in intratumoral stroma. Moreover, a significant positive correlation between MerTK protein expression and the amount of stroma was observed by immunohistochemistry ($Rho = 0.462$, $p < 0.001$). These data were further confirmed in a public iCCA dataset (n = 78) showing that high MerTK levels are associated with immunologically hot iCCA samples as indicated by tumor infiltration, immune activation, elevated expression immune checkpoint molecules. Single-cell RNA sequencing of CD45+ cells from non-tumoral and tumoral areas in iCCA patients showed that MerTK is predominantly expressed at the level of myeloid cells. Further reclustering showed MerTK expression in ID3 MØs, corresponding to Kupffer cells in tumor tissue. Notably, expression of MerTK correlated with greater tumor size, tumor grade, microvascular invasion, and risk of recurrence in iCCA patients.

Conclusion: These data indicate that a cross-talk between MerTK-expressing cells in the stroma and iCCA cells results in increased malignant features. In patients, MerTK expression predicts tumor recurrence.

P04-07
Oval cells as new players of the inflammatory response during cholestatic liver damage: role of the EGFR pathway

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Background and aims: During chronic cholestatic damage, toxic levels of bile acids accumulate in the liver causing cell death. In turn, bile acid accumulation is associated with reactive bile duct proliferation, a process called ductular reaction. This process involves different cell populations, including hepatic progenitor cells (known as oval cells/OC in rodents) that proliferate in the damaged liver and can differentiate to cholangiocytes and hepatocytes depending on the specific surrounding microenvironment. Despite their implication in the liver response to cholestatic injury how these cells respond to bile acids or cholestatic agents has not been well characterized, being the main purpose of this study.

Method: We used a model of mouse OC line. Cholestatic damage was simulated in vitro by treating OC with different bile acids alone or combined at different concentrations. Cell viability and death were tested, together with activation of signaling pathways and regulation of gene expression by RT-qPCR. The role of the EGFR pathway was analyzed by co-treatment with EGFR ligands and/or treatment with an EGFR inhibitor. For the cell-cell crosstalk studies we used conditioned media derived from OC to treat the GRX mouse stellate cell line.

Results: Our results show that some bile acids can trigger a cytotoxic effect in OC that involves either apoptosis and/or necrosis. Since we and others have demonstrated a key role for the Epidermal Growth Factor Receptor (EGFR) signaling pathway during liver regeneration and the cellular response to cholestatic damage, we have used different approaches to analyze whether EGF signaling pathway is a critical component of the OC response to bile acids. Data prove that EGF impairs bile acid cytotoxicity in certain conditions, and interestingly, it synergizes with bile acids to promote an inflammatory response. Thus, both bile acid and EGF independently regulate the expression of chemokines and cytokines such as Cxcl1, Cxcl2 and IL6, and the co-treatment results in a synergistic up-regulation; while EGFR inhibition blocks the induction of inflammatory signaling pathways by bile acids. Furthermore, EGF and bile acid co-treatment regulates NLRP3 inflammasome activation in OC. Interestingly, OC-derived conditioned medium in the presence or not of bile acids and EGF significantly impacts on the hepatic stellate cell activation and/or proliferation. The effect of OC-derived conditioned medium on macrophage cell population is being studied.

Conclusion: Overall, we provide evidence supporting an active role for OC during cholestatic damage, which involves both an induction of an inflammatory response and an activation of hepatic stellate cells. We also demonstrate a key contribution for EGFR pathway in these responses.

P04-09
Bile duct injury in mdr2 knockout mice can be repaired with n-acetylcysteine treatment

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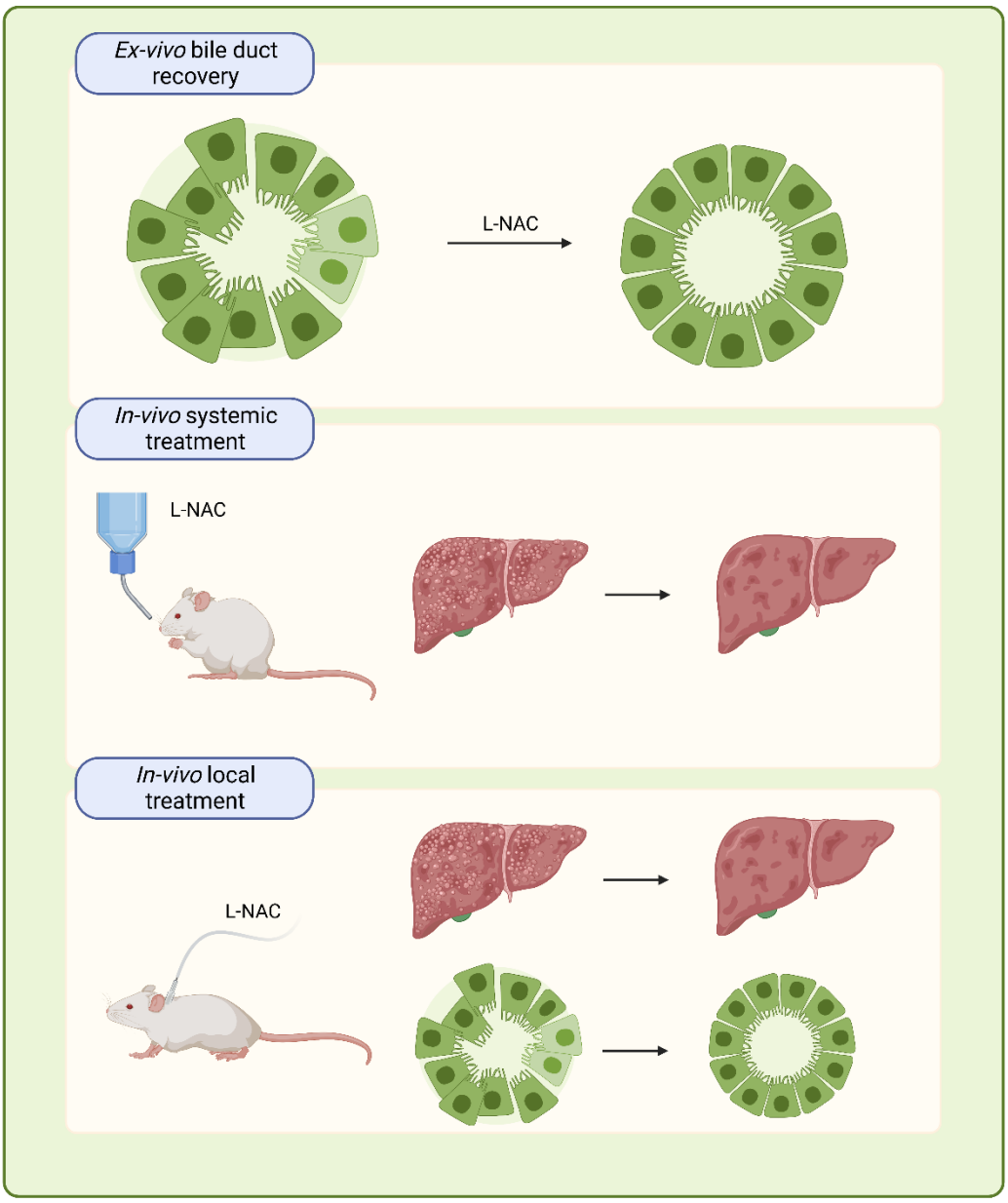
Background and aims: Primary sclerosing cholangitis (PSC) is a poorly understood cholangiopathy. Extrahepatic biliary tree phenotype is under studied both in the human disease and Mdr2$^{-/-}$ mice. In this study, we aimed to characterize extra hepatic biliary tree of Mdr2$^{-/-}$ mice and evaluate if the injury can be repaired with N-Acetyl-L-Cysteine (L-NAC), an antioxidant and glutathione precursor.

Method: We histologically characterized the extra hepatic biliary tree of Mdr2$^{-/-}$ mice, at various ages compared to wild type (WT) mice. Extrahepatic bile ducts (EHBDs) from 2 and 16-week old Mdr2-/- mice were dissected and incubated *ex-vivo* with or without the addition of 5µM L-NAC for 24 hours. EHBDs were stained for HandE, and immunofluorescent stained for K19 (cholangiocyte maker), ZO1 (apical tight junction), Vimentin (myofibroblst marker), and DAPI. In addition, we supplemented the drinking water of 16 and 24 weeks old Mdr2$^{-/-}$ mice with 30gr/L L-NAC, and assessed mice liver and EHBD response to L-NAC, compared to WT mice, by histological staining and serum liver enzymes level. Next, we combined local L-NAC treatment in a whole animal model by inserting bile duct catheters into the mice gallbladders, and injected the mice twice a day with 50µM L-NAC.

Results: Mdr2$^{-/-}$ mice developed progressive EHBD injury over time with progressive luminal complexity and reactive epithelial changes, including nuclear crowding, focal pseudo stratification, and epithelial sloughing, together with mild periductal inflammation which resembled human PCS pathology. In addition, *ex-vivo* local L-NAC treatment significantly improved EHBD morphology both at 2 and 16 weeks of age with improvement in lumen shape and nuclear crowding. Tight junctions which are already impaired at 2 weeks of age, as noted by absence of ZO-1 stain compared to WT were restored with L-NAC treatment at 2 weeks old Mdr2$^{-/-}$ EHBDs but not 16 weeks old, in addition to reduction in Vimentin levels, indicating restoration of tight junctions in early damage. Interestingly, Mdr2$^{-/-}$ mice treated with L-NAC added to their drinking water, showed significant improvement in liver enzymes levels, along with substantial reduction in intrahepatic periductal fibrosis, with a reduction of 40% "onion skin" on histology, and 60% reduction in quantitative Sirius Red stain, although the EHBD morphology improvement was not significant. Combining local L-NAC treatment in a whole animal using bile duct catheter resulted with significant improvement in liver enzyme along with EHBD recovery.

Conclusion: Mdr2$^{-/-}$ mice have progressive EHBD disease mimicking human PSC EHBD disease. Injured EHBD have a potential for recovery, mainly at early stages of injury. Oral administration of L-NAC has a mild effect on liver injury, though limited effect on EHBD; Local L-NAC treatment using bile duct catheters resulted in both biochemical improvement and EHBD recovery

Figure:


Ex-vivo bile duct recovery
L-NAC
In-vivo systemic treatment
L-NAC
In-vivo local treatment
L-NAC

P04-10-YI
Increased protein tyrosine phosphatase non-receptor type 2 activity in primary sclerosing cholangitis

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Background and aims: Primary sclerosing cholangitis (PSC) is a chronic liver disease characterized by multifocal biliary strictures. PSC is closely associated with ulcerative colitis (UC). The protein tyrosine phosphatase non-receptor type 2 (PTPN2) gene risk variant rs1893217 is associated with a severer disease course in inflammatory bowel disease (IBD) and PTPN2 was mentioned as a possible risk gene also for PSC. PTPN2 is involved in nod-like receptor protein (NLRP) 3 inflammasome assembly. NLRP3 activation triggers cholangitis in mice and was found in patients with PSC. This study explores if PTPN2 in PSC livers is activated and could trigger cholangitis.

Method: PTPN2 expression and activity was assessed in livers of patients suffering from PSC (N = 13) with and without (N = 6) UC and patients with liver cirrhosis due to other diseases, such as alcoholic liver disease (N = 9). Snap frozen liver tissue was used for the phosphatase activity assay and formalin fixed liver sections were stained using immunohistochemistry to assess the intrahepatic PTPN2 levels.
Results: Protein expression of PTPN2 in PSC and non-PSC livers was similar. However, the PTPN2 phosphatase activity was higher in PSC livers compared to non-PSC.

Conclusion: We detected higher PTPN2 activity in human PSC livers. If this is linked to NLRP3 activation and bile duct destruction needs to be elucidated.

Figure:

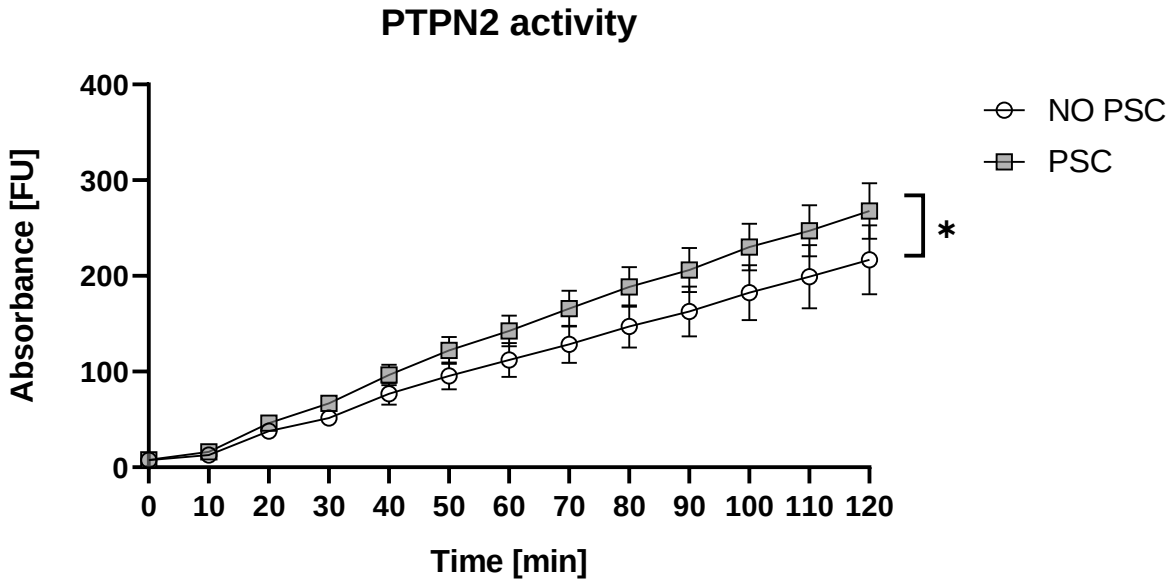

Figure 1: Enhanced PTPN2 phosphatase activity in PSC patients. PTPN2 phosphatase activity in non-PSC (N = 9) and PSC (N = 13) samples. The absorbance was normalized to the amount of protein (measured with Western Blot). End point Two-tailed Mann Whitney test, p = 0.0364

P05-02-YI
scRNA transcriptomics analysis of cellular cross talk in fibrocystin-defective mice revelas a central role for cholangiocytes, neutrophils and double-negative T cells

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Background and aims: Congenital hepatic fibrosis (CHF) and Caroli disease (CD) are caused by mutation in Polycystic kidney hepatic disease 1 gene (PKHD1) and lead to biliary malformations, cholangiocyte dysfunction and portal fibrosis. PKHD1 encodes for fibropolycystin (FPC), a protein expressed in cilia, plasma membrane and centromeres of cholangiocytes. FPC is involved in multiple cellular functions from polarity and cell matrix interactions to differentiation. This study was designed to investigate the relationships among the cell types present in the pericystic infiltrate in Pkhd1-KO mice at single cell level, to better understand the pathophysiology of CHF/CD.

Method: We isolated single cells from liver portal tract of 3 months old Pkhd1-KO and WT mice. Transcriptomics profiling of 16, 383 single cells, yielded molecular definitions for cell types present in samples. Datasets were analyzed by Seurat and CellPhoneDB package in R.

Results: ScRNA seq analysis revealed 9 distinct populations in the portal tract of WT and Pkhd1-KO. Interestingly, cholangiocytes in Pkhd1-KO have higher expression of chemokines (Cxlc1, Cxcl5). Gene ontology analysis of cholangiocytes showed an enrichment of genes involved in the recruitment of neutrophils and activation of innate immune responses and suggested the potential involvement of microbial components. CellPhoneDB analysis showed that Cxcl1 and Cxcl5 and the Cxcr2 receptor may play a major role in cross talk and recruitment of neutrophils by cholangiocytes. Neutrophils also express Ccl6, that signals to macrophages and T-cell through Ccr1 and Ccr2. Furthermore, neutrophils express Csf1 that promotes macrophage polarization. Among 4 different sub-populations of T cells identified, Double Negative T Cells (DNT) were detected only in Pkhd1-KO sample. DNT cells express IL17A and are believed to have a critical role in perpetuating inflammation. IL17A interacts with IL17RC receptor in cholangiocytes, and also with IL17RA in neutrophils possibly leading to further amplification of the of the inflammatory response. Macrophages express Cd86 that provide signals for naive T cell activation and survival by binding to Cd28 on CD4 + and CD8+ T cells.

Conclusion: This study shows a complex signaling network originating from cholangiocytes and amplified by the recruited neutrophils and then T cells and macrophages in Pkhd1-KO. Many of the identified signals are druggable and therefore their blockade may bear therapeutic advantages to improve inflammation and progression to fibrosis in Pkhd1-KO model. The potential role of microbial populations is of particular interest.

P05-03-YI
Claudin-1 is a therapeutic target for primary sclerosing cholangitis

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Background and aims: Primary Sclerosing Cholangitis (PSC) is a chronic cholestatic disease characterized by peribiliary fibrosis leading to biliary strictures, end-stage liver disease and cholangiocarcinoma. Clinical care of PSC patients lacks effective therapeutic strategies other than liver transplantation. Claudin-1 (CLDN1) is a transmembrane protein involved in epithelial tight junctions and is also expressed non-junctionally mediating cell plasticity and signaling. We have previously developed a highly specific monoclonal antibody (mAb) targeting exposed CLDN1 outside the tight junctions exhibiting an excellent safety profile in non-human primates. The aim of this study was to investigate the functional role of CLDN1 as a therapeutic target for PSC.

Method: CLDN1 expression in liver tissues of patients was analysed using scRNAseq, immunohistochemistry, and multicolor immunofluorescence. Proof-of-concept studies using CLDN1-specific mAbs were performed in the DDC mouse model, a well-recognized animal model for PSC. Fibrosis was assessed by collagen and fibronectin staining of liver tissues. The therapeutic effect on the phenotypes of the different cell populations of the hepatobiliary system was assessed by snRNAseq and immunohistochemistry.

Results: In tissues of patients with advanced liver disease, CLDN1 was highly expressed by EPCAM+ progenitor cells as well as cholangiocytes. Immunohistochemistry of PSC patient-derived liver tissues revealed up-regulated CLDN1 expression in cholangiocytes, reactive ductular biliary epithelial cells, and peri-portal hepatocytes, as well as fibroblasts and macrophages. Proof-of-concept studies in the DDC mouse model showed that a three-week treatment with anti-CLDN1 mAb reduced cholestasis as shown by decreased alkaline phosphatase levels. Moreover, anti-CLDN1 mAb markedly and significantly reduced hepatobiliary fibrosis and its porto-portal progression without detectable adverse effects.

Conclusion: Our results suggest a functional role of CLDN1 in the pathogenesis of PSC and provide preclinical proof-of-concept for a CLDN1-specific mAb as a novel therapeutic approach for PSC.

P05-04-YI
Dimethyl-fumarate attenuates cholestatic liver injury in mice

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Background and aims: Cholestatic liver diseases are characterized by intrahepatic accumulation of bile acids (BAs), resulting in liver inflammation, atypical ductular proliferation, and liver fibrosis. So far, protective features have been described as agents that alleviate BA overload and suppress inflammation. Dimethylfumarate (DMF) is an immunomodulatory drug approved for the treatment of inflammatory diseases such as psoriasis and multiple sclerosis. Therefore, we hypothesized that DMF might ameliorate the course of experimental cholestasis.

Method: Male C57Bl/6J mice were placed on a diet containing 3, 5-Diethoxycarbonyl-1, 4-Dihydrocollidine (DDC) for four weeks. Simultaneously, mice were treated with daily oral gavage of DMF (25 mg/kg) for four weeks while continuing the diet. At the end of the feeding period, stool, blood, bile, liver, and small intestine were harvested. Samples were analyzed by LC-MS for individual bile acids. In addition, immunohistochemistry, qRT-PCR, and Western blot were used to evaluate molecules related to bile acid homeostasis and liver injury. Human hepatic stellate cells (hHSC) were treated with TGF beta 1 and DMF (50 uM) for 24h hours. Cells were harvested, and mRNA was analyzed.

Results: As expected, the DDC diet promoted liver inflammation, ductular reaction, and liver fibrosis in parallel with elevated BA liver content and plasma concentrations. On the other hand, DDC-fed mice showed reduced BA biliary secretion, fecal excretion, and small intestine content. Interestingly, DMF reduced plasma transaminases, improved histological features of liver fibrosis, and decreased liver mRNA expression of *Coll1a1*, *Coll1a2*, and *Ccl2* in DDC mice compared to the untreated group. Moreover, DMF increased the fecal excretion of BAs, reducing their concentrations in plasma and liver content. In vitro, DMF selectively prevented hHSC activation measured by *Acta2* and *Coll1a1* mRNA levels.

Conclusion: Taken together, dimethyl-fumarate suppresses liver inflammation and liver fibrosis in the DDC-induced model of chronic cholestasis. Moreover, dimethyl-fumarate alleviates the retention of BAs by their reduced reabsorption in the ileum in the DDC model. Our data indicate that dimethyl-fumarate may be salutary for further testing in human fibrosing liver disorders.

P05-05
The tight junction protein jam-a is crucial for biliary pathophysiology in mice

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Background and aims: The Bile Acid (BA) receptor TGR5 regulates biliary epithelial barrier function through an impact on the tight junction protein JAM-A expression and phosphorylation, thereby protecting liver parenchyma against bile leakage during obstructive experimental liver diseases. Our efforts now focus on the importance of JAM-A in cholestatic liver diseases.

Method: We used wild type (WT) and JAM-A deficient (JAM-A$^{-/-}$) mice and measured their epithelial paracellular permeability by injecting fluorescent dextran either in the gallbladder (GB) (biliary epithelial permeability), or intravenously followed by bile analysis. WT and JAM-A$^{-/-}$ mice were submitted to BA-overload models: ● DDC (3, 5-diethoxycarbonyl-1, 4-dihydrocollidine) diet, ● cholic acid (CA)-enriched diet, ● bile duct ligation (BDL) w/or w/o cholecystectomy (CC)). In this context, we studied the following parameters on liver (and GB) sections: ● inflammatory cell infiltration, ● cell proliferation (KI67 labelling), ● liver injury (plasma liver enzymes, hepatocyte (hep.) necrosis), and ultimately ● survival rates over 14 days.

Results: *In vivo* paracellular permeability was significantly increased in JAM-A$^{-/-}$ as compared with WT mice, in both hep. and cholangiocyte (chol.) compartments.
In the DDC model, mimicking progressive cholestasis w/obstructive component as seen in human PSC, we demonstrated that JAM-A$^{-/-}$ mice submitted to 1 week of DDC showed significantly: ● more inflammation, ● exacerbated ductular reaction (chol. proliferation) and ● more liver injury, as compared to WT mice.
In the CA-enriched diet model, mimicking BA overload with a shift toward a hydrophobic BA pool composition, JAM-A$^{-/-}$ mice fed with 1% CA during 2w exhibited increased: ● inflammation (Gr1 antibody, granulocyte and macrophage marker) and ● cell proliferation on liver (hep. and chol.) and gallbladder (chol.) sections, as compared to WT mice.
In the BDL model, mimicking either acute or chronic obstructive cholestasis, we observed: ● an increased gallbladder weight associated with an increased epithelial cell proliferation, and ● a strongly increased (80% vs 20%) mortality rate in JAM-A$^{-/-}$ mice compared to WT mice over a 14d period.
Interestingly in the BDL model, GB weight increase and epithelial cell proliferation were correlated with bile retention volume in the GB in WT mice, while this correlation was deeply altered in JAM-A$^{-/-}$ mice.

Conclusion: In each of the different biliary models used, the lack of JAM-A was robustly associated with a worsened phenotype highly suggesting that JAM-A is of crucial importance in hepatobiliary pathophysiology. JAMA appeared to modulate both the inflammatory and ductular responses to injury in the liver. Our preliminary data suggest that JAM-A could operate as a mechanosensor in liver epithelial cells.

P05-09-YI
Biliary senescence affects the transcriptomic landscape of murine hepatocytes

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Background and aims: Biliary diseases are often characterised by biliary epithelial cell (BEC) senescence which, together with its senescence-associated secretory phenotype (SASP), is proposed to regulate liver injury. However, the effects of BEC senescence and its SASP on the modulation of hepatocyte biology remain unexplored. Here, we aimed to generate an in vitro model of BEC senescence and study its impact on hepatocyte transcription dynamics.

Method: A murine primary BEC line (Ep-CAM+CD24+CD133+) was treated with etoposide (a DNA topoisomerase inhibitor) to induce senescence, which was assessed via microscopy and RT-qPCR. Conditioned medium from senescent BECs was subsequently added to naïve BECs to confirm transmitted BEC senescence. A non-contact insert-based system was then used to co-culture senescent BECs and naïve primary hepatocytes. Bulk RNA-seq and transcriptomic analysis were performed to assess the effects of the BEC SASP in hepatocytes.

Results: Senescence and SASP in etoposide-treated BECs were confirmed by a significant increase of senescence-associated beta-galactosidase activity and gene expression of *Cdkn1a*, *Trp53bp1*, *Il6*, *Ccl2*, *Cxcl2*, *Cx3cl1*, *Serpine1*, *Vegfa* and *Pdgfa*. These were accompanied by a decrease in proliferation (EdU fluorescence and *Mki67* gene expression). The secretome of senescent BECs promoted secondary senescence and SASP-related gene expression in naïve BECs, including significantly increased *Cdkn1a*, *Cdkn1b*, *Cx3cl1* and *Vegfa*. Differential expression and gene set enrichment analyses of RNA-seq data from treated BEC donors confirmed the presence of senescence and SASP-related factors. In hepatocytes exposed to senescent BECs, differential expression of RNA-seq data revealed a significant increase of the SASP-related factor *Vcam1*. Functional enrichment analysis using the STRING database retrieved the highest enriched terms (false discovery rate [FDR] <0.0001): (1) decreased “fatty acid omega-oxidation” (enrichment score [ES] 7.65); (2) increased “neutrophil extravasation, and myeloperoxidase” (ES 6.16); and (3) increased “cathelicidin, and neutrophil aggregation” (ES 5.56). Additional terms (FDR <0.01) included decreased “drug metabolism-cytochrome P450” (ES 4.01); increased “leukocyte migration involved in inflammatory response” (ES 2.58); and increased “regulation of chemokine production” (ES 1.57).

Conclusion: Murine BEC senescence was modelled in vitro. In our experimental setting, senescent BECs present SASP features typical of human biliary diseases. Our model revealed several BEC-dependent SASP-induced changes in hepatocytes that reflect structural, metabolic and functional alterations. Together, this data suggests that, in the context of biliary disease, hepatocytes exposed to senescent BECs display decreased metabolism and enhanced immunomodulatory abilities.

P05-10
Extracellular matrix remodeling in the progression of primary sclerosing cholangitis to cholangiocarcinoma: insights from an animal model

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Background and aims: Primary Sclerosing Cholangitis (PSC) is a chronic biliary disease characterized by peribiliary fibrosis evolving to cholangiocarcinoma (CCA). As PSC, CCA is characterized by prominent extracellular matrix (ECM) accumulation. Despite this association, the mechanisms by which fibrosis is accompanied by dysplasia and malignant transformation remain uncharted, reflecting the lack of an appropriate experimental model to capture the sequence from PSC to CCA (CCA/PSC). In this study, we aimed at developing an animal model of CCA/PSC to assess changes in ECM proteins typically up-regulated in CCA (Osteopontin (OPN), Tenascin C (TnC) and Periostin (POSTN)), as potential biomarkers enabling early detection of tumoral lesions in PSC.

Method: Mrd2$^{-/-}$ mice, a well-established PSC model, were treated with thioacetamide (TAA), a profibrogenic toxicant, to induce CCA, and sacrificed at different time points (12 and 28 weeks, both n = 4) and compared with controls (Mdr2$^{-/-}$ without TTA and WT+TAA mice, both n = 4). ECM phenotyping was performed in liver sections from mice and human archival samples of CCA/PSC (n = 5) by immunohistochemistry (IHC) for OPN, TnC and POSTN. *In vitro*, human CCA cells (HUCCT-1, EGI-1) and primary human PSC cells were treated for 24h with OPN (10nM), TnC (100nM), and POSTN (100nM) to evaluate cell viability (MTS), migration (wound healing), and cancer stem cell (CSC)-like phenotypic switch (CD133 and CD44, real-time PCR).

Results: Upon TTA treatment, Mdr2$^{-/-}$ mice but not controls developed biliary dysplasia (2/4 at 12, 1/4 at 28 weeks) and intrahepatic CCA (1/4 at 12, 3/4 at 28 weeks). IHC showed a progressive increase from classic ductal to dysplastic and neoplastic lesions for TnC, POSTN, and more prominently for OPN, similarly to what seen in human samples. *In vitro,* 24h treatment with ECM proteins enhanced migratory properties in CCA, but not in PSC cells. POSTN, and less efficiently TnC and OPN, induced a significant upregulation of CD133 and CD44 in both CCA and PSC cells, signature of CSC-like phenotype.

Conclusion: The Mdr2$^{-/-}$+TAA mouse recapitulates the pathogenetic sequence biliary fibrosis-biliary dysplasia-CCA. In both murine and human samples OPN, TnC and POSTN are overexpressed in malignant lesions. *In vitro*, ECM proteins, particularly POSTN, induce a CSC-like switch in CCA and PSC cells, in support of their tumor-initiating role. ECM proteins may serve as putative tissue biomarkers for CCA surveillance in PSC patients.

POSTER ABSTRACT PRESENTATIONS

Clinical Science

P01-03
Quantitative MRCP differentiates between malignant and benign biliary obstructions

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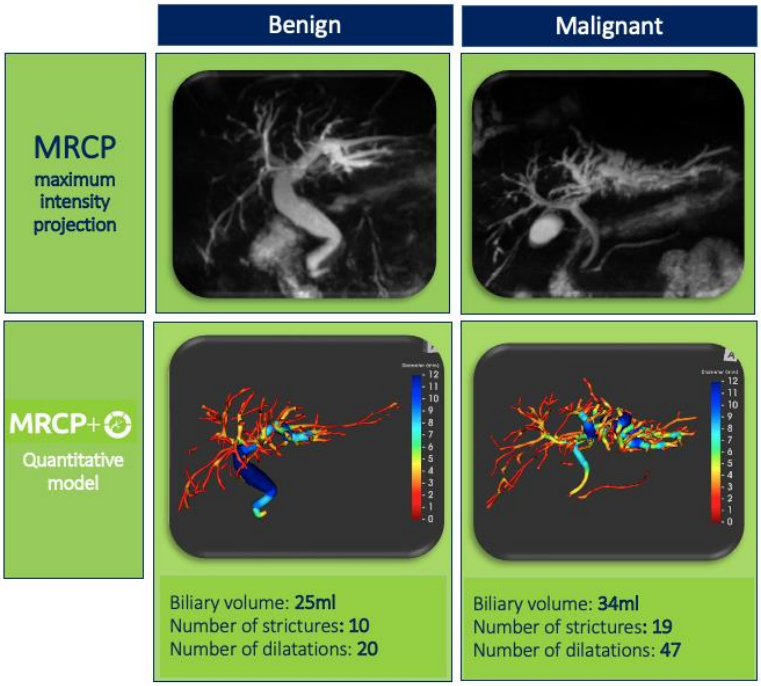
Background and aims: Cholangiocarcinoma (CCA) is a rare cancer with a prevalence of around 3 in 100, 000 people. If detected at an early-stage, curative surgical resection is associated with a 5-year survival of up to 35% compared to <12 months when detected at late stage. However, due to difficulty differentiating benign from malignant biliary obstruction using traditional methods, detection of CCA remains a challenge. Recently, Quantitative MRCP (MRCP+™ Perspectum Ltd), a novel quantitative MR technique that automatically segments biliary anatomy from standard magnetic resonance cholangiopancreatography (MRCP) has been developed to provide quantitative measurements of the biliary tree. This study sought to determine whether Quantitative MRCP could be used to differentiate benign and malignant biliary obstructions.

Method: In this retrospective study of 37 patients [67.6% male, 65.3 ± 9.7 years], 21 patients with biopsy or surgery confirmed malignant obstructions, and 16 patients with benign biliary obstructions underwent standard MRCP imaging. MRCP images were post-processed with MRCP+ software which, using AI-driven pathfinding algorithms created a 3D model of the biliary tree. Measurements of total number of ducts, number of strictures, number of dilatations, total length of strictures and dilatations, total biliary tree volume and total length of ducts were calculated automatically from the derived model. A Mann-Whitney U test compared the metrics across malignant and benign groups. The prognostic potential of biliary volume calculated using Quantitative MRCP was assessed using linear regression.

Results: All bile duct metrics calculated using Quantitative MRCP were found to be significantly higher in malignant biliary obstruction ($p < 0.05$). Of the metrics assessed: total number of ducts ($p = 0.04$), total number of strictures ($p = 0.01$), total number of dilatations ($p = 0.001$), total length of ducts ($p = 0.002$), total length of strictures and dilatations ($p = 0.002$), the total volume of the biliary tree was found to be the most significant predictor of malignancy (AUC = 0.81; [95%CI 0.67-0.96], $p = 0.001$). A volume of ≥25ml was shown to significantly differentiate the two populations with a sensitivity of 85.7% and specificity of 68.8%. While overall accuracy of detecting malignant obstruction at a volume of ≥25ml was 78.4%.

Conclusion: Quantitative MRCP metrics, and in particular total biliary tree volume are shown here to differentiate malignant (CCA) from benign obstructions. Given that current pathways requires either contrast administration or ERCP, a procedure with associated increased risks of mortality and morbidity, quantitative MRCP metrics may offer an objective, non-invasive biomarker to identify CCA.

Figure: MRCP (top) and Quantitative MRCP (bottom). Quantitative MRCP differentiate between benign (left) and malignant (right) obstructions.



P01-10-YI
Dynamics of spleen stiffness measurement in patients with primary biliary cholangitis predict disease progression: a pilot study

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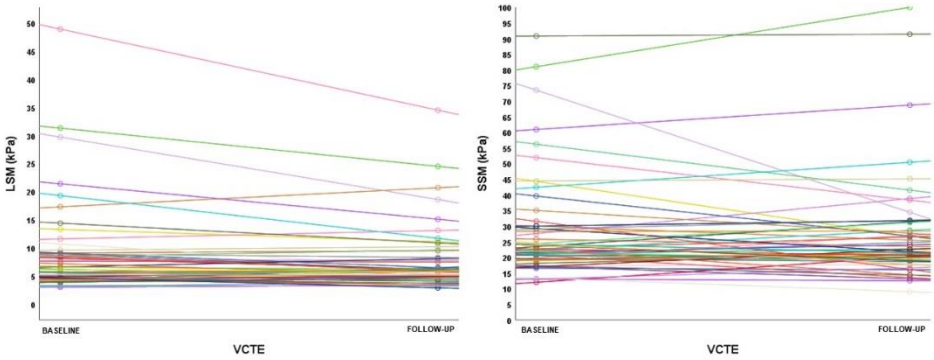
Background and aims: In primary biliary cholangitis (PBC) liver stiffness measurement (LSM) by vibration-controlled transient elastography (VCTE) is recommended at baseline for discrimination of early and advanced stage disease and can be repeated during treatment for risk stratification. Recently, spleen stiffness measurement (SSM) by VCTE has been proposed as a tool complementary to LSM to refine non-invasive stratification of risk of clinically significant portal hypertension and decompensation. We aimed to assess dynamics of SSM in PBC patients.

Method: Monocentric study of 100 PBC patients (96% women), who underwent VCTE for LSM and SSM with a spleen dedicated module. Among the 100 patients, 56 underwent two sequential longitudinal VCTE examinations for LSM and SSM (median time between the two examinations: 16 months). Changes in LSM and SSM during follow-up were defined as significant for ≥30% variation of baseline value. Demographic and clinical features at baseline and at the time of VCTE examination were recorded.

Results: Among the 100 patients: median age was 63 years (54-71), median disease duration 40 months (16-84), 13% were cirrhotic, 95% on monotherapy with ursodeoxycholic acid (UDCA), 5% on combination therapy with obeticholic acid, median LSM was 6.4 kPa (IQR 4.8-8.8), being ≥10 kPa in 16/100 (16%); median SSM was 21.9 kPa (IQR 19-29), being >40 kPa in 15/100 (15%). In the 56 patients who underwent repeat VCTE evaluation, median LSM and SSM did not change significantly between first and second examination. LSM remained stable in 45 (80%), increased in 4 (7%) and decreased in 7 (13%). SSM was stable in 45 (80%), increased in 5 (9%) and decreased in 6 (11%). None of the patients with stable LSM and SSM had liver-related events. In 3 of 4 patients in whom LSM increased, the value remained below 6.5 kPa; in 1 patient LSM increased from 9.8 kPa to 13.1 kPa and SSM from 19.8 kPa to 27 kPa, follow-up liver biopsy showed development of cirrhosis, while endoscopy revealed small esophageal varices. In 2 patients both LSM and SSM decreased: in 1 patient LSM decreased from 48 kPa to 33 kPa, SSM from 52 kPa to 37 kPa, follow-up endoscopy revealed disappearance of previously diagnosed small varices; one patient started UDCA therapy after the first VCTE examination.

Conclusion: This pilot study shows that in the majority of PBC treated patients LSM and SSM remain stable during follow-up. Changes of both LSM and SSM during follow-up might anticipate progression or improvement of liver disease, both in term of fibrosis and of clinical signs of portal hypertension.

Figure:


LSM (kPa)
BASELINE
FOLLOW-UP
VCTE
SSM (kPa)
BASELINE
FOLLOW-UP
VCTE

P02-05-YI
Transcriptomic risk score predicts response to obeticholic acid and identifies genes linked with metabolic remodelling, autophagy, senescence and fibrosis in patients with PBC

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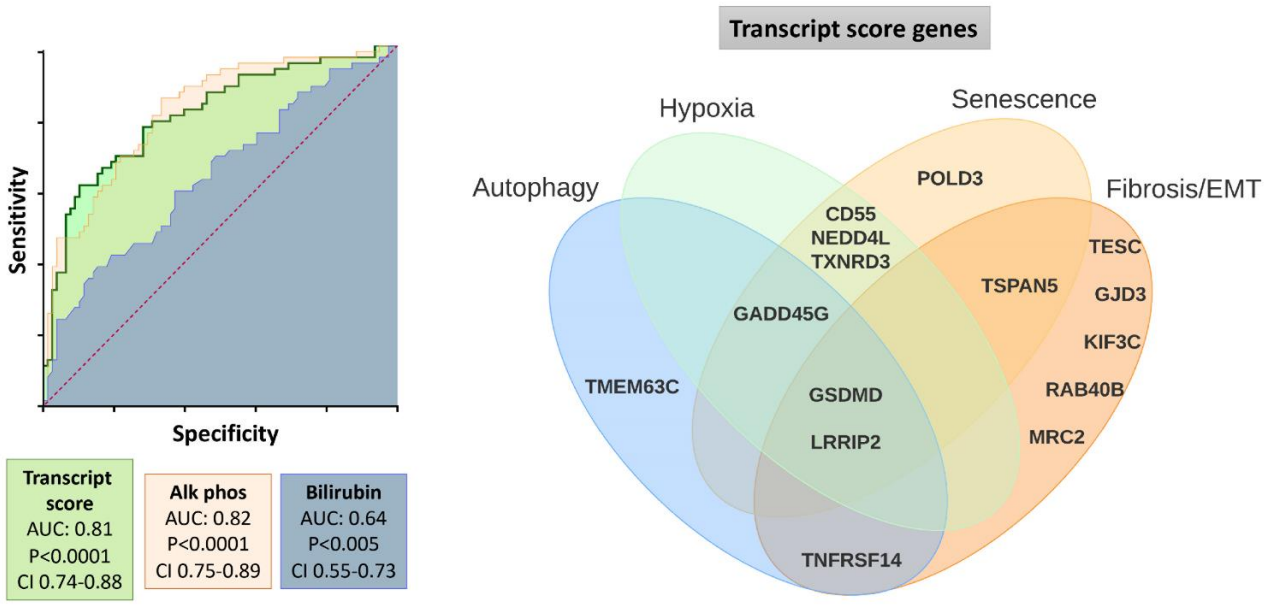
Background and aims: Globe and UK-PBC scores, histology and neutrophil to lymphocyte ratio (NLR) as a surrogate for lymphopenia (AASLD, 2021;763A) inform outcomes in PBC. As transcriptomic scores may predict response to treatment, we evaluated PBC patients enrolled in the POISE study prior to intervention by whole blood RNAseq and develop a prognostic score using the transcripts that correlated with clinical outcomes.

Method: A whole blood RNAseq database of serial samples from PBC patients was screened for differential gene expression using pre-treatment samples. Patients were stratified by either highest vs lowest alkaline phosphatase (ALP) (n = 11 each group) or NLR as an indicator of lymphopenia (n = 11 each group). Log transformed counts of dysregulated genes measured in transcripts per million were serially evaluated by (i) linear regression modelling versus serum ALP or bilirubin levels, (ii) non-parametric t-test with OCA responders versus non-responders using the POISE end point criteria, and (iii) OCA treatment response vs non-response by area under receiver operator characteristic (AUROC). Then, the final transcription score was constructed by adding/subtracting log transcripts in an unbiased fashion using machine learning. This predictive score was then compared with serum liver tests as a predictive biomarker.

Results: Serial assessment of over 5, 000 whole blood transcripts was performed using machine learning to derive a predictive score of 16 transcripts that either positively or negatively correlated with serum liver tests [positive: TSPAN5, NEDD4L, SLC6A9, MRC2, TMEM63C, GSDMD, TNFRSF14, GADD45G, GJD3, TESC; negative: POLD3, TXNRD3, CD55, KIF3C, RAB40B, LRRFIP2]. The 16 gene transcriptomic score demonstrated a positive correlation with ALP (R^2 = 0.12, $p < 0.0001$), bilirubin (R^2 = 0.12, $p < 0.0001$) and distinguished OCA responders and non-responders (median score: 4.6 vs 6.0 respectively, $p < 0.0001$). The transcriptomic score was found to have an AUROC value of 0.81, which was comparable with 0.82 for ALP and superior to 0.64 for bilirubin (Figure, left). Predictive transcripts were evaluated for biological processes previously linked with the development of fibrosis in PBC cholangiocytes (Figure, right) and all but one of the transcripts (SLC6A9) were associated with one or more of these processes: metabolic remodeling with autophagy (n = 5), hypoxia (n = 7), DNA damage and senescence (n = 8) and fibrosis/epithelial to mesenchymal transition (n = 9).

Conclusion: In PBC patients treated OCA, the transcriptomic score demonstrated a similar capacity to stratify and predict disease as serum ALP levels. Further longitudinal studies incorporating measurement of liver stiffness will be required to determine whether these findings are generalizable to other treatments and if the transcriptomic score reflects disease activity predictive of progressive fibrosis.

Figure:


Sensitivity
Specificity
Transcript score
AUC: 0.81
P<0.0001
CI 0.74-0.88
Alk phos
AUC: 0.82
P<0.0001
CI 0.75-0.89
Bilirubin
AUC: 0.64
P<0.005
CI 0.55-0.73
Transcript score genes
Hypoxia
Senescence
Autophagy
Fibrosis/EMT
POLD3
CD55
NEDD4L
TXNRD3
TESC
TSPAN5
GJD3
GADD45G
KIF3C
TMEM63C
GSDMD
RAB40B
LRRIP2
MRC2
TNFRSF14

P03-08-YI
Non-invasive evaluation of progression to primary biliary cholangitis in patients with positive antimitochondrial antibodies

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Background and aims: Only a low percentage of people with positive antimitochondrial antibodies (AMA+) develop primary biliary cholangitis (PBC). However, there is no tool to predict who will and what their management should be. Here, we aim to evaluate the usefulness of the serum bile acid (BA) profile as a non-invasive follow-up tool in AMA+ subjects.

Method: Longitudinal study in AMA+ individuals identified in the ETHON cohort (multicenter population-based study) between 2015-2017 in Spain, including transient elastography (TE) data. Twenty BA species and 7alpha-hydroxy-4-cholesten-3-one (C4) were analyzed in serum samples by mass spectrometry (HPLC-MS/MS), both at inclusion and follow-up. Patients with a diagnosis of PBC during recruitment were excluded.

Results: Of the 12, 246 individuals included in the ETHON cohort, 9, 655 underwent AMA testing. Fifty-four AMA+ individuals without PBC were identified, of whom 38 (73%) were women with a median age of 49.5 years, all of them having normal liver blood tests and TE <8 kPa. The majority (47 subjects, 87%) of the initial cohort continued follow-up for a median of 5.3 years (IQR 4.8-5.8). Six patients (12.7%) developed PBC. The BA profile of non-progressors (NOPROG) *vs.* those who progressed to PBC (PROG) was assessed at both baseline and follow-up visit (median time 4.8 years, IQR 4.7-5). At baseline, PROG individuals compared to NOPROG had higher concentrations of non-12-alpha-hydroxylated BAs (non-12aOH) (2.49 *vs.* 1.45 uM; p = 0.04) and glycoconjugated BAs (1.75 *vs.* 1.03 uM; p = 0.06), as well as lower proportion of cholic acid (CA) species in the serum BA pool (9.9 *vs.* 17.1% p = 0.02). At follow-up, PROG patients compared to NOPROG presented higher concentrations of 12aOH (1.75 *vs.* 0.69 uM; p = 0.01), non-12aOH (2.00 *vs.* 0.84 uM; p = 0.04), glycoconjugated (1.86 *vs.* 0.69 uM; p = 0.05) and free (1.05 *vs.* 0.49 uM; p = 0.07) BAs. In paired analysis, PROG patients had increased concentrations and proportion of molecular species of the CA (p = 0.02 and p = 0.04, respectively) and deoxycholic acid (p = 0.07) families, an increase that was also reflected in the concentration of 12aOH BAs. Moreover, concentrations of non-12aOH BAs in PROG patients remained elevated and stable (p = 0.91). On the other hand, in NOPROG subjects, the profile of all BAs species remained stable during follow-up, showing no alterations in serum BA concentrations or proportions.

Conclusion: AMA+ individuals without progression to PBC show a stable and non-pathological serum BA profile, whereas those who progress show significant changes in their BA profile. Patients who progress show at baseline significantly higher levels of non-12aOH BAs than those who do not progress, which may be a useful early prognostic biomarker for the development of PBC.

P03-09-YI
Primary sclerosing cholangitis with features of autoimmune hepatitis: phenotypic characterization and prognosis

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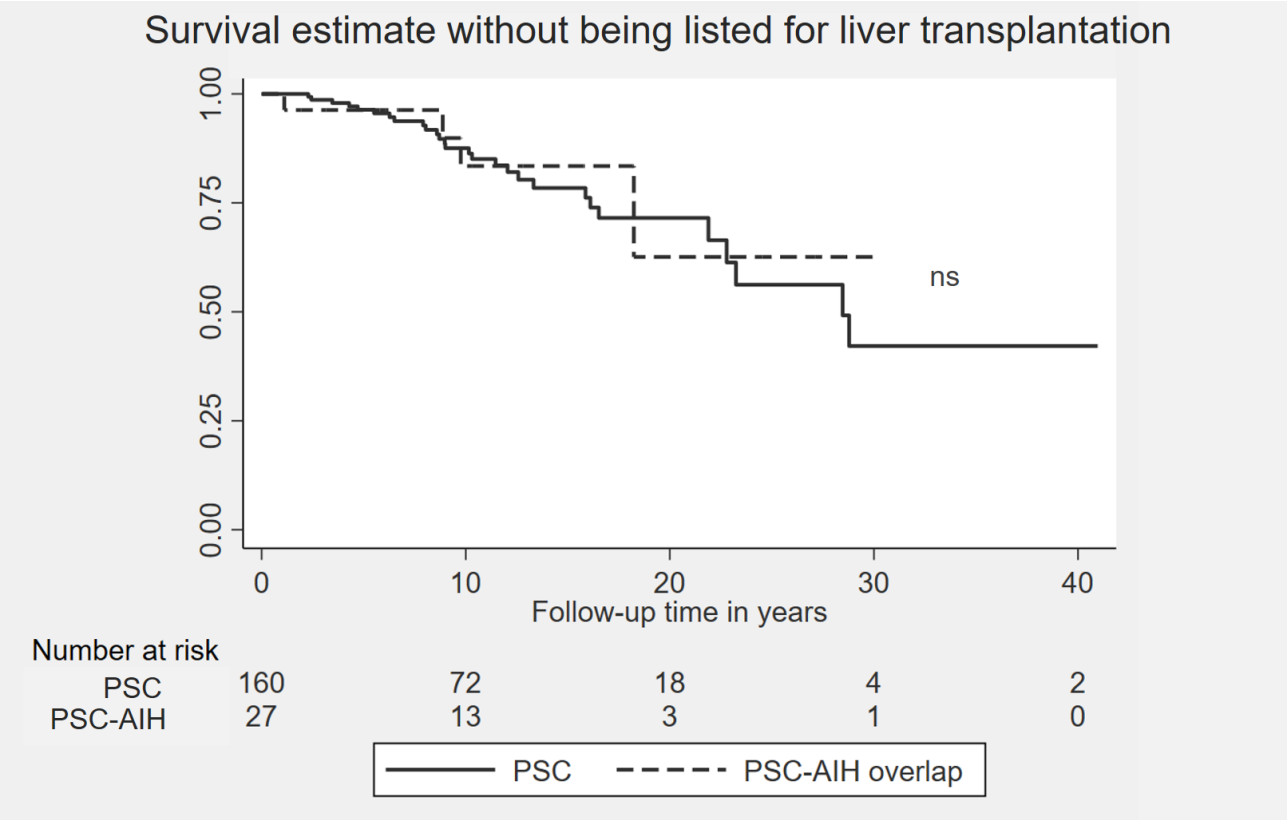
Background and aims: A minority of patients with primary sclerosing cholangitis (PSC) display features of autoimmune hepatitis (AIH), which is usually called PSC-AIH overlap syndrome (OS). The prognosis of OS is poorly known. The aim of this study was to compare the prognosis of OS patients to the prognosis of PSC patients.

Method: This retrospective analysis included OS and PSC patients followed in our tertiary referral center for cholestatic diseases from 1995 to 2023 with at least one year of follow-up. OS was defined by the presence of two out of three criteria for AIH (ALAT >5xupper limit of normal; IgG ≥1.5 ULN or smooth muscle antibody titer >1:80; typical/compatible signs on liver histology); histology was mandatory. The primary outcome was survival without being listed for liver transplantation (LT) and was assessed using log-rank test and adjusted restricted mean survival time (RMST) analysis.

Results: 27 patients with OS and 160 with PSC were included. Median age at diagnosis was 21 years (15-28) in OS and 29 years (21-43) in PSC (p = 0.002). 70.4% were male in the OS group and 73.1% in the PSC group (p = 0.8). Small duct PSC was present in 14.8% of the OS group and in 8.8% of the PSC group (p = 0.3). Inflammatory bowel disease was present in 44.4% of OS patients and in 75% of PSC patients (p = 0.002). All patients received ursodeoxycholic acid. Every OS patient received immunosuppressive treatment (prednisolone (in 89% of patients), azathioprine (92.9%), budesonide (10.7%), mycophenolate mofetil (14.3%) and calcineurin inhibitors (8.3%)). Cirrhosis at diagnosis was present in 29.6% of OS patients versus 10.8% of PSC patients (p = 0.01). At last follow-up, cirrhosis was reported in 44.4% of OS and in 30.8% of PSC patients (p = 0.19). Median follow-up time was 8.87 years (5.8-15.2) in OS and 6.5 years (3.37-11.3) in PSC patients. Eight patients (5%) in the PSC group developed cholangiocarcinoma, in comparison to none in the OS group. Listing for LT was necessary in four patients (14.8%) in the OS group and in 23 patients (14.4%) in the PSC group (p = 1). Five patients (3.1%) in the PSC group died from a liver related cause, none died in the OS group (p = 0.65). Survival rates were not significantly different between OS and PSC patients (Figure), even after adjustment for sex, age and cirrhosis at diagnosis.

Conclusion: Compared to patients with PSC, OS patients display a younger age at diagnosis and a less common association with inflammatory bowel disease. Although they display a more advanced liver disease at diagnosis, OS patients treated with immunosuppressive treatment have a survival similar to the one of PSC patients.

Figure: Kaplan Meyer estimate for survival without being listed for liver transplantation



P04-01
Chronic ketamine-induced sclerosing cholangitis associated with early inflammatory bowel disease after long term topical use

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Background and aims: Ketamine is a safe general anesthetic. It is metabolized in the liver by microsomal cytochrome enzymes and the metabolites are excreted in urine. Long-term recreational abuse and prolonged therapeutic ICU use has been associated with ketamine-induced sclerosing cholangitis (KISC). We report KISC after prolonged topical use for peripheral neuropathy. Uniquely, there was associated mucosal changes in the colon suggestive of early inflammatory bowel disease.

Method: Case Summary-A 38-year-old female presented to the ED in 9/2021 with RUQ pain and elevated AlkP. Ultrasound showed multiple small and large stones in gallbladder. A laparoscopic cholecystectomy was performed for cholecystitis.She had a history of Alport syndrome, with two prior renal transplants. Multiple LFTs, including AlkP, documented between 8/2018 and 3/2021 had been completely normal (N <113 iu/ml). There had been a slow increase in AlkP starting from 4/2021 (354 u/L) to the time of presentation, when AlkP was 985 u/L and GGT 1060 u/L (N <38 iu/ml). Between 9/2021 and 11/2022, she had multiple admissions for epigastric pain with nausea and vomiting. Serum bilirubin has remained normal. Serological workup, including ANA, AMA and IgG4 were unrevealing, as were routine viral tests. EBV and CMV infections were ruled out.
She admitted to regular use of compounded ketamine and lidocaine lotion topically over several months for peripheral neuropathy.

Results: A liver biopsy performed 4/2022, showed expansion of portal tracts with edema, bile ductular proliferation, neutrophilic inflammation and focal bile duct injury with focal periportal fibrosis. Immunohistochemical stain for CMV was negative. MRCP showed abnormal peribiliary restricted diffusion with corresponding increased peribiliary T2 signal, consistent with cholangitis. ERCP with balloon cholangiography showed no intraluminal filling defects. Peripheral ducts were pruned, suggestive of small duct sclerosing cholangitis.

A colonoscopy for hematochezia noted skip lesions in the colon. Biopsies of the rectal mucosa showed focal ulceration with severe activity and fibrinopurulent exudate. The ascending colon showed focally, severely active ulcerated mucosa with increased lamina propria eosinophils (up to 75 per HPF). The differential diagnosis included infection, drug or medication effect or early inflammatory bowel disease.

Conclusion: Our case illustrates KISC after topical long-term use, uniquely associated with early inflammatory bowel disease.

P04-06-YI
Biliary atrasia human cholangiocyte organoids demonstrate increased oxidative stress response

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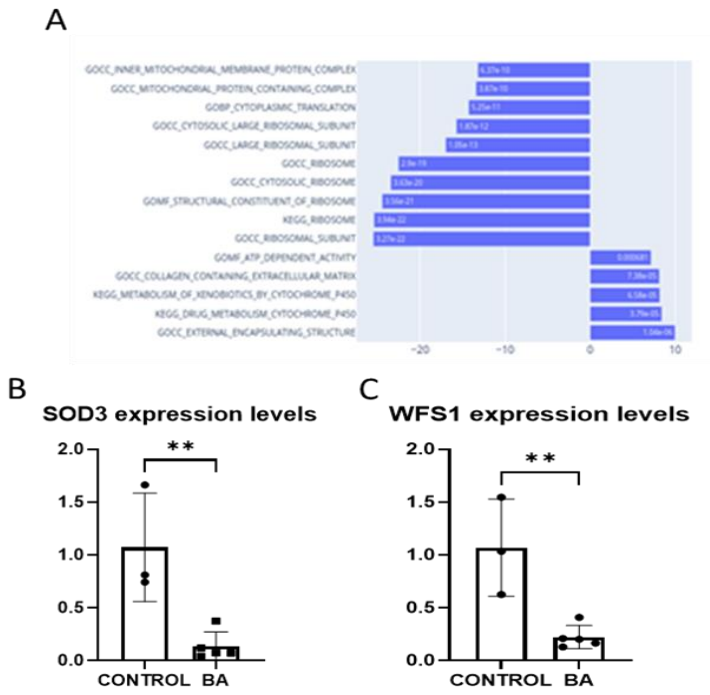
Background and aims: Biliary atresia (BA) is a form of biliary fibrosis typically diagnosed in previously healthy newborns before the age of 3 months, with extrahepatic bile duct obstruction and progressive liver fibrosis. The etiology of the disease is unknown. Here we aimed to identify the pathophysiology of the primary cholangiocyte injury in human BA and the potential for recovery, using bulk and single cell gene expression data from different BA patients.

Method: We cultured human cholangiocyte organoids (HCOs) derived from BA patients and non-BA controls, in order to identify pathways involved with BA cholangiocyte injury. Differentially expressed pathways were further studied using qPCR and western blotting in a candidate gene approach.

Results: BA derived HCOs display deformed shape and lumen obstruction. RNA sequencing results identified differentially expressed genes in BA HCOs involved in several biological pathways, including endoplasmic reticulum (ER) stress, unfolded protein response, generation of reactive oxygen species, and drug metabolism (Figure 1A). The results were validated at the RNA and protein levels. We used RT-PCR to compare differences in gene expression-related ER stress between BA patients and non-BA controls. Superoxide dismutase 3 (SOD3) is an extracellular antioxidant defense against oxidative damage, which deficiency induced spontaneous liver injury and fibrosis.SOD3 was downregulated by 7 fold ($p = 0.0067$) in BA patients compared to control (Figure1B). WFS1 is a component of the IRE1 and PERK signaling pathways which Negatively regulates the ER stress response, was downregulated by 5 fold ($p = 0.0062$) in BA patients compared to control (Figure1C).

Conclusion: BA derived HCOs are characterized by ER stress and unfolded protein response, which may be result in dysfunctional cell-to-cell adhesion. These findings shed light on mechanisms of injury in BA and may contribute to the discovery of potential therapies.

Figure:


A
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GOCC_CYTOSOLIC_LARGE_RIBOSOMAL_SUBUNIT
GOCC_LARGE_RIBOSOMAL_SUBUNIT
GOCC_RIBOSOME
GOCC_CYTOSOLIC_RIBOSOME
GOMF_STRUCTURAL_CONSTITUENT_OF_RIBOSOME
KEGG_RIBOSOME
GOCC_RIBOSOMAL_SUBUNIT
GOMF_ATP_DEPENDENT_ACTIVITY
GOCC_COLLAGEN_CONTAINING_EXTRACELLULAR_MATRIX
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450
KEGG_DRUG_METABOLISM_CYTOCHROME_P450
GOCC_EXTERNAL_ENCAPSULATING_STRUCTURE
-20
-10
0
10
B
SOD3 expression levels
**
2.0
1.5
1.0
0.5
0.0
CONTROL
BA
C
WFS1 expression levels
**
2.0
1.5
1.0
0.5
0.0
CONTROL
BA

P04-08-YI
Radiomics features for risk stratification in primary sclerosing cholangitis: a proof-of-concept study

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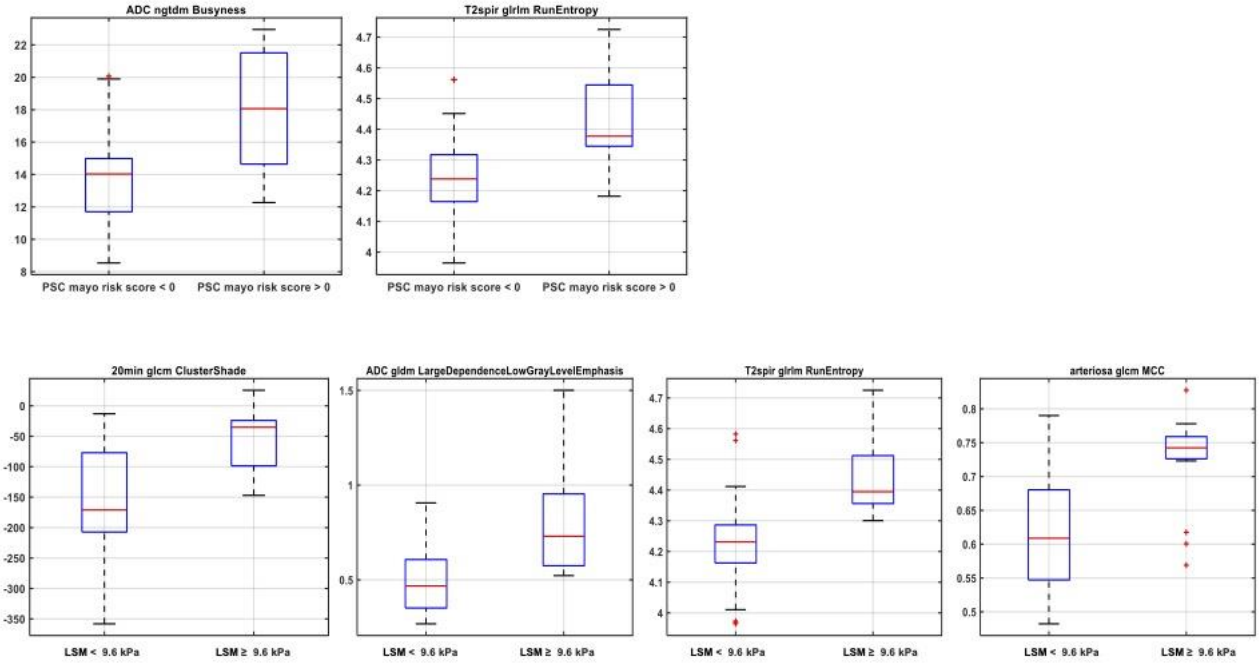
Background and aims: Magnetic resonance cholangiopancreatography (MRI-MRCP) assessment in primary sclerosing cholangitis (PSC) is currently based on qualitative or semi-quantitative parameters and has high inter-observer variability. A quantitative, reproducible evaluation of radiological biomarkers might improve their use in risk stratification. Aim of the study was to identify radiomics features semi-automatically extracted from MRI-MRCP images associated with high risk of clinical outcome.

Method: Fifty-eight PSC patients who underwent routine gadoxetate disodium-enhanced MRI-MRCP acquired with a standardized protocol were prospectively enrolled from Jan-2020 to Dec-2021. Blood tests and liver stiffness measurement were collected close to the MRI-MRCP. Patients were classified into high risk for disease progression using either the Mayo risk score (MRS) and liver stiffness measurement (LSM). Radiomics features have been extracted using PyRadiomics in each of the five MRI-MRCP sequences analyzed.

Results: Among the 58 patients, 15 (25.0%) and 17 (30.0%) were considered at high-risk using MRS and LSM, respectively. One hundred and seven radiomics features have been extracted from each MRI-MRCP sequence analyzed. The selection process identified two features associated with MRS>0: *NGTDM-Busyness* in the ADC and *GLRLM-Run Entropy* in T2spir showing both a mean cross-validated AUC of 80% (figure). The multivariable model, including both the features, showed a mean AUC of 87% (SD 11%). When considering LSM (>9.6Kpa) as a stratifier of disease severity the features selected were *GLCM-Cluster Shade* in T1W HBP phase, *GLCM-Maximal Correlation Coefficient* in T1W arterial phase, *GLDM-Large Dependence Low Gray Level Emphasis* in ADC, and *GLRLM-Run Entropy* in T2spir with AUC of 85%, 83%, 85%, and 92%, respectively (figure). The most accurate multivariable model included three variables: *GLDM-Large Dependence Low Gray Level Emphasis* in ADC, *GLRLM-Run Entropy* in T2spir and *GLCM-Cluster Shade* in T1W HBP phase with a mean AUC of 96%.

Conclusion: This proof-of-concept study highlighted the potential of semi-automatically acquired radiomics features for risk stratification in PSC. Validation toward clinical end points on a larger cohort are warranted.

Figure:


ADC ngtdm Busyness
PSC mayo risk score < 0
PSC mayo risk score > 0
T2spir glrlm RunEntropy
PSC mayo risk score < 0
PSC mayo risk score > 0
20min glcm ClusterShade
LSM < 9.6 kPa
LSM ≥ 9.6 kPa
ADC gldm LargeDependenceLowGrayLevelEmphasis
LSM < 9.6 kPa
LSM ≥ 9.6 kPa
T2spir glrlm RunEntropy
LSM < 9.6 kPa
LSM ≥ 9.6 kPa
arteriosa glcm MCC
LSM < 9.6 kPa
LSM ≥ 9.6 kPa

P05-01-YI
Long-term treatment with fibrates and ursodeoxycholic acid in people with primary sclerosing cholangitis is safe and associated with persistent clinical and biochemical improvement

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Background and aims: People with Primary Sclerosing Cholangitis (PSC) have no effective medical treatment for delaying disease progression. Ursodeoxycholic acid (UDCA) improves liver tests (LFTs) and prognostic markers. Fibrates, PPARs agonists, are recommended as first-line therapy for pruritus in PSC. However, long-term data on safety and effectiveness of fibrates on pruritus, LFTs and prognostic markers in PSC are lacking. Aim of this study was to assess long-term safety and effectiveness of fibrates in PSC.

Method: Retrospectively, we collected data of PSC people treated with fenofibrate (200 mg/day) or bezafibrate (400 mg/day) for at least 6 months in addition to UDCA, for persistent alkaline phosphatase (ALP) elevation (>1.5xULN), pruritus or dyslipidemia. Changes in LFTs, liver stiffness by VCTE, symptoms, prognostic scores and occurrence of adverse events every 6 months after fibrates introduction were collected.

Results: Twenty-seven consecutive PSC people who started fibrates (fenofibrate n = 25, bezafibrate n = 2) between 2017 and 2020 were included. Median age at diagnosis was 31 (21-35). Upon treatment with fibrates (median duration of 33.3 [20.8-51.2] months), we observed a significant reduction of ALP, GGT and transaminases. A decrease of ALP levels of 41%, 52%, 46%, 54% at 6, 12, 18, 24 months compared to baseline values, respectively, was observed. Nearly a third of people achieved ALP normalization at semestral evaluations. The Amsterdam-Oxford score significantly improved after 12 months (1.75*vs.*1.35, $p = 0.03$). Liver stiffness showed a non-significant reduction on long-term. Number of patients experiencing pruritus significantly decreased at each time point, as well as fatigue at 18 months ($p = 0.03$). One third of people interrupted fibrates for non-response, but no adverse events related to fibrate treatment occurred during follow-up.

Conclusion: Long-term combination of fibrates and UDCA in people with PSC is safe and associated with persistent clinical and biochemical improvement. While awaiting the results of ongoing phase 3 study, we consider fibrates as a valid therapeutic option in people with PSC and elevated liver tests despite UDCA**.**.

P05-06-YI
Recurrence of primary sclerosing cholangitis after liver transplantation: a single center data

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Background and aims: Primary sclerosing cholangitis (PSC) is a progressive cholestatic disorder, accounting for 5-15% of liver transplantation (LT) indications in Europe. A major drawback of LT results for PSC is recurrence of PSC (rPSC), observed in up to 25% of recipients. The aim of this study was to define rPSC rate and long-term outcomes after LT in a single center, and to identify potentially modifiable risk factors of rPSC.

Method: We performed a retrospective analysis of all consecutive patients who underwent a first LT for PSC over a 28-year period (1993-2021). Only patients with at least one-year post-LT follow-up were included. Recurrent PSC was diagnosed using previously published Mayo Clinic cholangiographic or histologic criteria. We collected data at waiting list (liver disease, inflammatory bowel disease (IBD) associated), at LT (donor features, type of surgery, pathological examination of the explant) and during the follow-up after LT (immunosuppressive regimen; cancer; evolution of IBD if associated), and if rPSC recurred. Patient and graft survival were reported using Kaplan-Meier method.

Results: Thirty-three patients who underwent LT for PSC were included. Median age at LT was 44 years (31.3-56.7), 17 (52%) were female, 24 patients (73%) had IBD associated. Twenty-three patients (70%) underwent LT for decompensated cirrhosis, 9 patients (27%) for recurrent cholangitis and 1 patient (3%) for perihilar cholangiocarcinoma (pCCA). Median donor age was 52 years (26.5-68.6), median cold ischemia time (CIT) was 8 hours (6.3-9.0). Nine patients (27%) developed rPSC during a median follow-up 59 months (IQR 45-72), with no significant difference across recipient gender and age. In univariate analysis, longer CIT ($p = 0.026$), donor female gender ($p = 0.049$), IBD reactivation post-LT ($p = 0.005$) and biliodigestive anastomosis at LT ($p = 0.019$) were associated with higher risk of rPSC. Six patients were listed for re-LT and 2 patients (6%) underwent re-LT. We observed graft survival of 94%, 86%, and 74% and patient survival of 97%, 89%, and 77% at 1, 5, and 10 years after LT, respectively, with no significant differences between rPSC and no-rPSC groups.

Conclusion: The identification of predictive factors for rPSC is challenging to avoid graft loss. Some donor and surgical features might increase rPSC risk. IBD reactivation might have a pathogenic role in rPSC. In our single center experience, rPSC did not affect patient and graft survival.

P05-07-YI
Hepatic recompensation upon treatment with ursodeoxycholic acid in PBC patients with decompensated cirrhosis

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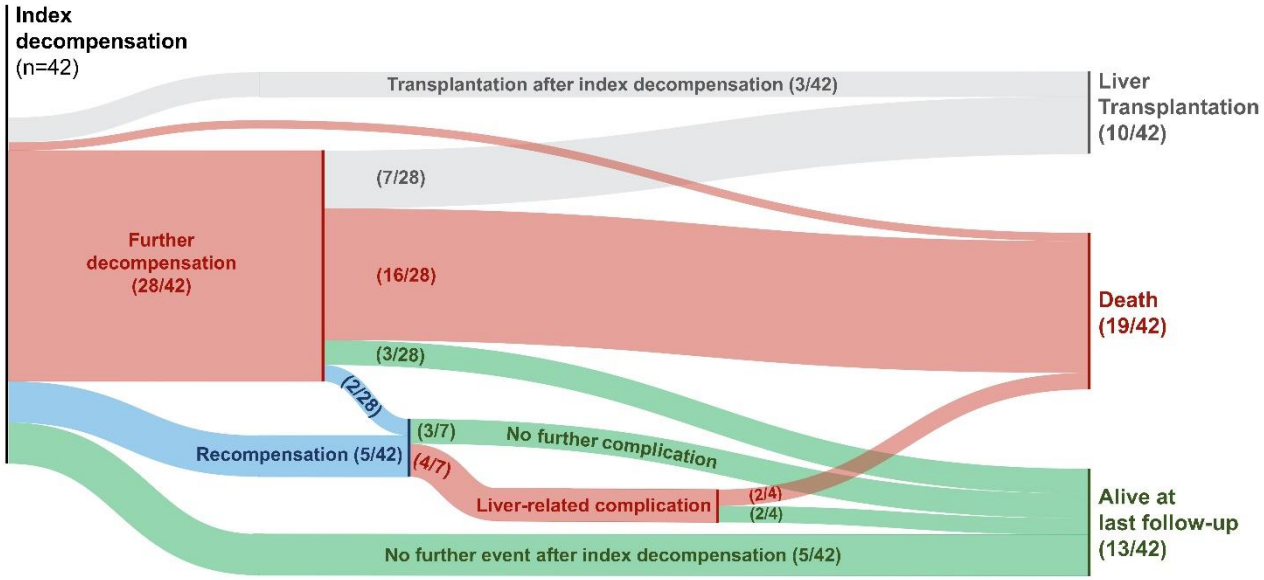
Background and aims: Patients with primary biliary cholangitis (PBC) are at risk of developing cirrhosis and subsequent hepatic decompensation which often necessitates liver transplantation. For other causes of liver disease, it has been documented that successful aetiologic therapy can ameliorate liver function and even induce hepatic recompensation. Here, we assessed the Baveno VII concept of recompensation in PBC, considering response to ursodeoxycholic acid (UDCA) according to Paris-II criteria as surrogate for successful aetiological therapy.

Method: We included PBC patients with decompensated cirrhosis (ascites, hepatic encephalopathy [HE], variceal bleeding) and assessed their further disease course following index decompensation. Recompensation was defined as resolution of ascites and HE, absence of variceal bleeding and sustained improvements in Child-Pugh and MELD-Na score.

Results: 42 PBC patients (age: 63.5 [IQR: 51.9-69.2] years; 88.1% female) with decompensated cirrhosis (ascites 54.8%, variceal bleeding 35.7%, HE 9.5%) and a median MELD-Na of 14 (11-15) were included. 33 patients received UDCA (median dose: 12.5 [IQR: 10.7-15.0] mg/kg BW) for at least 1 year and 36% (12/33) fulfilled Paris-II response criteria. During a median follow-up of 41.9 (11.0-70.9) months, 7 patients (16.7%) achieved recompensation with a mean decrease in Child-Pugh and MELD-Na score of 1 (p = 0.095) and 2.9 points (p = 0.041), respectively. Protective factors linked to a higher likelihood of recompensation in a competing risk model were variceal bleeding as index decompensation (SHR: 4.37; p = 0.069), a lower Child score (SHR: 0.59; p = 0.019), lower MELD-Na (SHR: 0.90; 0.047), lower bilirubin (SHR: 0.44; p = 0.005) and lower alkaline phosphatase (SHR: 0.96; p = 0.001). Interestingly, recompensation occurred in 5/12 (42%) UDCA responders, but also in 2/21 (10%) patients without UDCA response. Of the 35 patients who remained decompensated, 17 died (49%) and 10 underwent liver transplantation (29%). In a time-dependent cox regression model, transplant-free survival was numerically longer in patients achieving hepatic recompensation (HR for death: 0.46; p = 0.335). Importantly, 3 recompensated patients survived without liver transplantation over a follow-up time of 42, 71 and 332 months after index decompensation. Nevertheless, 4 recompensated patients developed hepatic complications related to hepatic malignancy and/or portal vein thrombosis (PVT). (Figure)

Conclusion: PBC patients with decompensated cirrhosis may achieve hepatic recompensation under UDCA treatment, especially if liver dysfunction is less pronounced at index decompensation. However, recompensated patients remain at risk for liver cancer and PVT and should thus be monitored closely since liver transplantation may still be necessary.
Figure: Sankey plot depicting the natural history of decompensated PBC


Index
decompensation
(n=42)
Transplantation after index decompensation (3/42)
Liver
Transplantation
(10/42)
(7/28)
Further
decompensation
(28/42)
(16/28)
Death
(19/42)
(3/28)
(2/28)
(3/7)
No further complication
Recompensation (5/42)
(4/7)
Liver-related complication
(2/4)
(2/4)
Alive at
last follow-up
(13/42)
No further event after index decompensation (5/42)

P05-08
Liver re-transplantation for recurrent primary sclerosing cholangitis: how far we can go?

Chiara Mazzarelli[1], Andrea Della Penna[2], Marco Carbone[3], Vincent Karam[4], Rene Adam[4], Costantino Fondevila[5], Luca Saverio Belli[1], Silvio Nadalin[2]

[1]ASST Great Metropolitan Niguarda, Hepatology and Gastroenterology, Milano, Italy, *[2]Tübingen University Hospital, Department of General, Visceral and Transplant Surgery, Tübingen, Germany*, *[3]University of Milan Bicocca, Gastroenterology, Monza, Italy*, *[4]Hôpital Paul-Brousse Ap-Hp, Centre Hépatobiliaire, Villejuif, France*, *[5]La Paz University Hospital, General and Digestive Surgery Service, Madrid, Spain*

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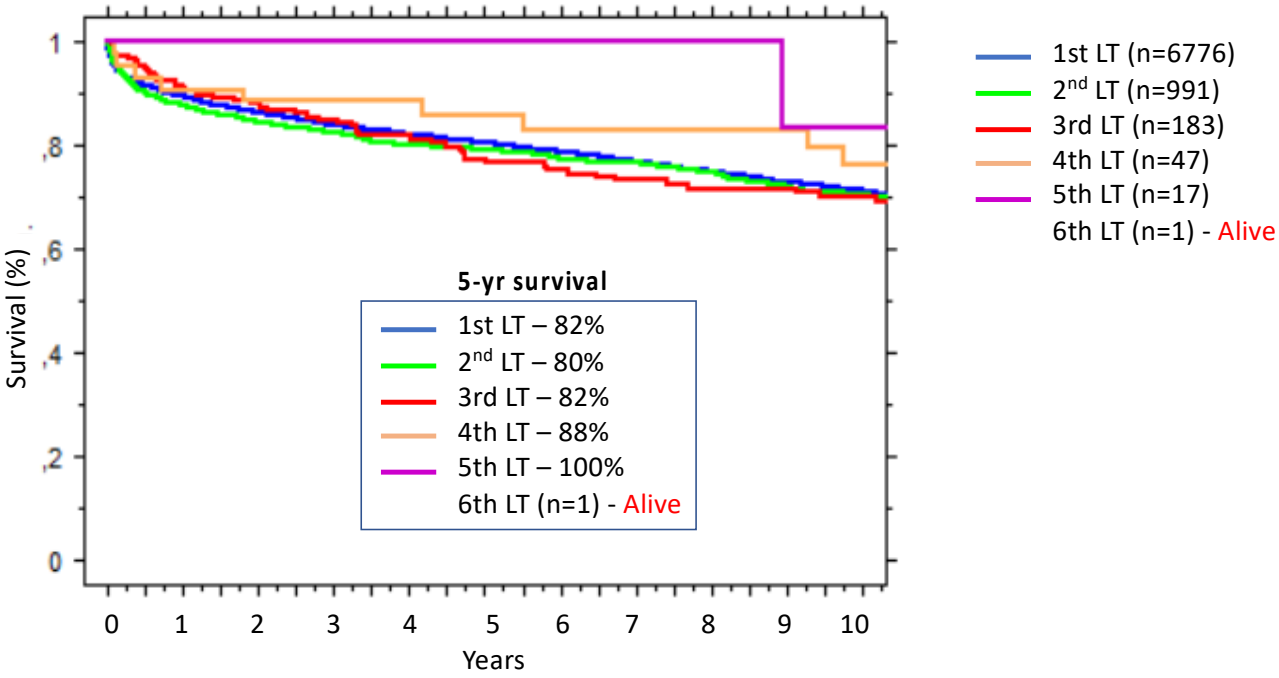
Background and aims: It has been reported that more than 25% of patients undergoing LT for PSC are affected of PSC recurrence (rPSC). It is still matter of debate if and how often a re-LT should be performed in such patients with such high risk of disease recurrence and graft loss. Aim of the present study was to provide an adequate answer to the dilemma above by analysing the results of retransplantation for rPSC from the European Liver Transplant Registry (ELTR) database.

Method: Retrospective analysis of data from the ELTR in the period from 1980 to 2021. Kaplan-Meier overall survival analysis was conducted with stratification of recipients according to the rank of LT.

Results: Out of 160, 460 LT performed during the study period, 8023 (5%) were for PSC, with a median age of 43 and 68% of recipients being male. A total of 991 patients underwent a second LT (12% of PSC recipients), 183 received a third transplant (2.3% of PSC recipients), 47 received a fourth transplant (0.6% of PSC recipients), and 17 received a fifth transplant (0.2% of PSC recipients), while only one patient received a sixth transplant. There were no significant differences in the survival curves for patients undergoing more than two liver transplants (Figure 1).

Conclusion: In the context of the absence of alternative therapeutic options, LT for rPSC remains a viable option, also in terms of utility, as survival after re-transplantation is not significantly different. However, the selection of patients who may benefit from more than one re-LT remains an open question.

Figure:



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