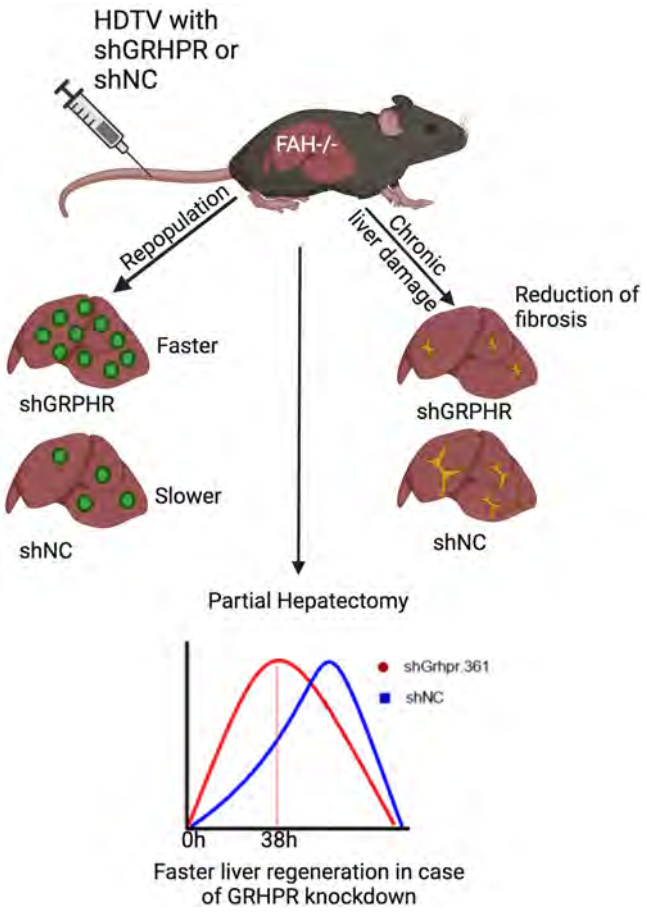
control. In addition, we observed that GRHPR knockdown in hepatocytes in vivo does not lead to tumor formation for 1 year.



Conclusion: Hepatocyte-specific knockdown of GRHPR improves liver regeneration and attenuates liver fibrosis. GRHPR knockdown in hepatocytes seems to be safe regarding liver cancer. We did not see any macroscopic impact on the kidney and mice survived for more than one year with hepatocyte-specific GRHPR knockdown. Nevertheless, kidney pathology has to be further evaluated.

SAT155

Expression and function of axon guidance genes in the developing portal tract

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Background and aims: The liver lobules consist of hepatocyte cords and sinusoids that radiate from the portal area to the central vein. The portal area contains a branch of the portal vein, a hepatic artery, a bile duct lined by cholangiocytes, lymphatics, nerves and a heterogeneous mesenchyme. During development, the portal mesenchyme instructs differentiation of cholangiocytes, and normal development of the bile ducts is required for hepatic arteriogenesis (Lemaigre, 2020). Yet, how portal mesenchyme, bile ducts and hepatic artery interact during development remains poorly understood. To address this issue, we hypothesize that the 3D morphogenesis of bile ducts and hepatic artery is guided by signaling interactions with mesenchymal cells in the portal tract.
Method: To investigate this hypothesis, we separately FACS-purified cholangiocytes and adjacent mesenchymal cells at various stages of liver development in mouse embryos, and subjected the cells to RNAseq analysis. We identified several ligand-receptor pairs where the ligand is differentially expressed in the mesenchyme and the receptor in the cholangiocytes, or vice versa, during duct morphogenesis. These included axon guidance genes, namely genes coding for Slit/Robo proteins, Eph receptors/Ephrins, Semaphorins/Plexins and Netrins/netrin receptors.
Results: We determined the cell-specific and spatial expression of selected genes, and focused our functional analyses on Slit/Robo and Netrin/Netrin receptor pairs due to their preferential location of expression near forming ducts (Fig. 1). Using a Cre-loxP strategy we inactivated selected genes in the developing mesenchyme or cholangiocytes. Functional assays, 2D and 3D imaging identified axon guidance genes required for normal development of bile ducts and hepatic artery.

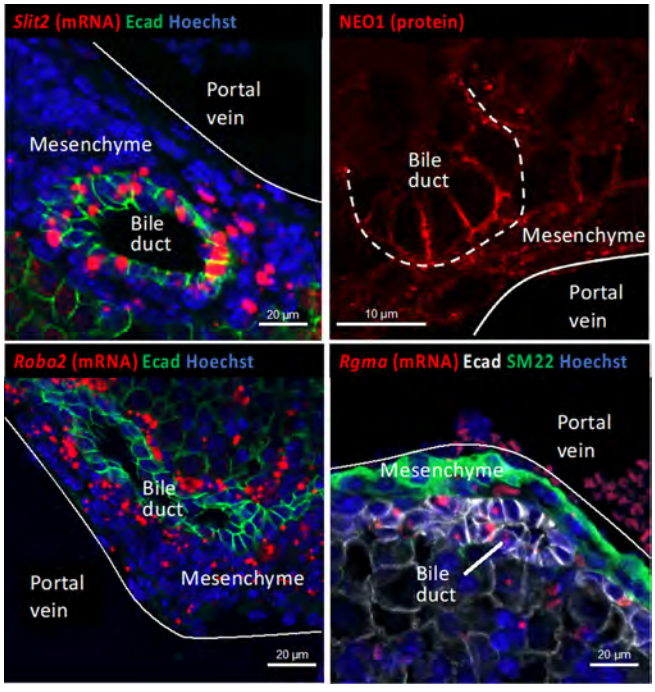

Figure: Expression of axon guidance genes (red) in developing mouse liver at E18.5.

Conclusion: We conclude that axon guidance pathways drive cell-cell interactions that control normal development of bile ducts and hepatic artery in the portal tract.

SAT156

Patients with chronic liver diseases are at risk for diabetes even before development of cirrhosis

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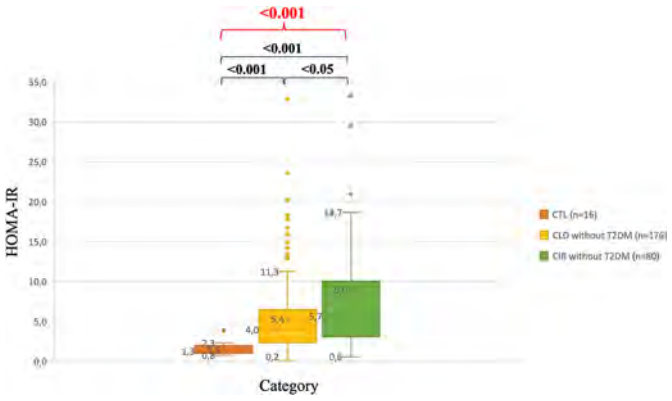
Background and aims: The prevalence of insulin resistance (IR) and type 2 diabetes mellitus (T2DM) is higher in patients with cirrhosis, compared to control patients without liver disease. The exact mechanism for this is unknown but could include liver inflammation. In this study, we investigate whether cirrhosis is the *primum movens* of IR or if impaired insulin sensitivity is already present in non-cirrhotic patients with chronic liver diseases.
Method: Patients were recruited and divided into three groups: control (CTL), active chronic liver disease without cirrhosis (CLD) and cirrhosis (CIR). In patients not taking pharmacological treatment for

T2DM, IR was quantified using the homeostasis model assessment of insulin resistance (HOMA-IR). The proportion of patients treated for T2DM was recorded in each group. Additionally, HOMA-IR levels among different disease etiologies were compared. The study was approved by the local ethics committee.

Results: 422 patients were included in our study, 16 were controls, 278 had a CLD and 128 were cirrhotic (CIR). The causes of liver disease were as follows: metabolic-dysfunction associated fatty liver disease (MAFLD, n = 206), alcoholic liver disease (ALD, n = 117), active chronic hepatitis C (HCV, n = 59), other cause (n = 24). IR, represented by a HOMA-IR value exceeding 2.5, is already present in patients with non-cirrhotic CLD (median HOMA-IR 4.0). HOMA-IR levels lie between those seen in CTL (median HOMA-IR 1.3) and CIR patients (median HOMA-IR 5.7), with a statistically significant difference between the three groups (p value <0.001) (Figure). Median glycemia in the CLD and CIR groups is the same (98 mg/dL and 99 mg/dL respectively) but insulinemia is different (101.5 pmol/land 132.3 pmol/lrespectively) resulting in distinct HOMA-IR values in CLD and CIR patients (p = 0.018). Compared to CLD patients, patients in the CIR group are also characterised by a higher age, higher AST levels and a lower platelet count consistent with portal hypertension. The number of patients with T2DM is the same in the CLD and CIR groups (36.7 and 37.5% respectively). Finally, HOMA-IR levels differ according to disease etiology (p value <0.001): MAFLD and HCV associated liver disease are associated with higher levels of IR compared to ALD and other causes of CLD.



Conclusion: Chronic liver disease is already a predisposing factor to T2DM, regardless of the presence of cirrhosis. Certain disease etiologies are associated with more severe IR. Cirrhosis is a factor which in itself elicits additional increase in insulin levels. The increased insulin levels in this group may be related to increased IR in this situation or to decreased insulin clearance due to portal hypertension.

SAT157

Characterization of bioengineered liver constructs using hepatoblast organoids (HBOs) and human liver extracellular matrix (ECM) 3D-platforms

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Background and aims: Primary fetal hepatoblast organoids (HBOs) derived from human fetal livers have self-renewal ability and can be consecutively passaged while retaining capacity to differentiate to both the hepatic and biliary lineages. We hypothesized that the environment provided by the decellularised healthy human liver extracellular matrix (ECM) will offer HBOs the natural acellular cues for growth and enhanced metabolic functionality and maturation. Thus, the aim of this study was to demonstrate proof of concept for survival and growth of HBOs in a bio-compatible healthy human liver 3D-platform and assess their functionality.

Method: Healthy human liver unsuitable for transplantation was decellularized. Acellular liver tissue was lyophilized ≥24 hours and comminuted to a particulate form. ECM powder was then solubilised, neutralized, sterilised, and mixed with Nanocellulose (Celllink) 70:30 in the resultant ECMgel. HBO cells that had been cultured and expanded on Matrigel were seeded on ECMgel (25 K cells/30 μL). Standard HBO Matrigel cultures (25 K cells/20μL) were used as controls. ECMgel (n = 36) and Matrigel (n = 36) samples for RNA extraction/gene expression, live/dead staining or ELISAs were collected up to day 14.

Results: HBOs repopulated the Matrigel and ECM gel and remained viable for 14 days in maintenance medium. HBO clusters on ECMgel had similar size but displayed a distinctively round shape compared to Matrigel where organoids have a characteristic budding morphology. Albumin gene expression in ECMgel was significantly higher compared to Matrigel samples and showed a significant increase from day 7 to 14. aFP gene expression showed a decreasing trend from day 7 to 14 in both materials. SERPINA and OTC gene expression of HBOs in ECMgel vs Matrigel was also higher on day 14, while CYP3A4 was higher in ECMgel on both timepoints. AURK gene expression in ECMgel was significantly higher than in Matrigel on day 14, suggesting higher proliferation. KRT19 expression was lower on ECMgel on day 7 compared to Matrigel and ranged in very low levels in all samples. Secreted albumin/aFP ratio significantly increased from day 5 to 14 in ECMgel indicating improved maturation but not in Matrigel samples.

Conclusion: Primary HBOs can grow in a healthy human liver ECM 3D-platform *in vitro* for at least 2 weeks and start differentiating towards a hepatocyte phenotype with the signals provided solely by the healthy human liver ECM. Overall, by combining two novel bioengineering technologies we showed the feasibility of constructing *in vitro* small implantable liver tissues that could be used for *in vivo* transplantation in future studies.

SAT158

Novel avenue towards liver regeneration and treatment of acute and chronic liver diseases: safety and PK profile from a first-in human (FIH) clinical trial for HRX-0215 as first in class MKK4 inhibitor

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Background and aims: Impaired liver regeneration is a hallmark of acute and chronic liver diseases and to date liver transplantation still represents the only ultimate treatment for acute liver failure and end stage chronic liver diseases. Recent reports validated Mitogen Activated Protein (MAP) Kinase 4 (MKK 4) as a novel target and demonstrated a role as a master regulator of liver regeneration (Wuestefeld et al., Cell 2013). Particularly striking efficacy results