generated using liver resections obtained from UZ Brussel from which hepatocytes were freshly isolated with a Percoll gradient and HSCs (UV+), ECs (CD32+) and Kupffer cells (CD45+ CD163+) by FACS.
Results: Since we incorporate iHepatoblasts in spheroids that need to mature to iHeps and since HSCs play a crucial role in chronic liver diseases, we used iHep maturation and iHSC activation as readouts for the optimisation of cell ratios in the spheroids. This resulted in an optimised ratio of 2 iHSC : 1 iHepatoblast : 0.6 iEC : 0.6 iMφ. We compared differentiation towards mature iHeps in 3D co-cultures to differentiation in 2D iHep monocultures (21 days). We detected higher gene expression levels of mature hepatocyte markers at day 21 of spheroid cultures compared to 2D cultures. This suggests that differentiating iHeps in 3D in the presence of other liver cell types is beneficial for their maturation. To evaluate the liver cell types in co-culture spheroids further, we performed RNAseq at the start (day 1) and at the end (day 21) of spheroid cultures. GO analysis showed that day 1 cells were still actively in the cell cycle, while spheroids cultured for 21 days showed high expression of genes involved in metabolic processes, i.e. phase I and II metabolism, drug and bile acid metabolism and gluconeogenesis. Similar to Hep markers, EC (PECAM1) and Mφ (ITGAM) markers increased over time, suggesting further maturation of these cells in 3D as well. These findings were confirmed with immunofluorescence stainings on day 21 spheroids for CYP3A4, CD31, CD45 and PDGFRβ for Heps, ECs, iMφ and HSCs respectively. At day 21, spheroids showed decreased expression of HSC activation markers and increased expression of markers specific for quiescence, such as respectively COL1A1 and LRAT, suggesting that the iHSCs become more quiescent in these co-cultures. To mimic fibrosis, we either directly (TGF-β) or indirectly (APAP) induced iHSC activation. Both conditions led to a significant increase of HSC activation marker genes (ACTA2, COL1A1, LOXL2) indicating functionality of both iHSCs and iHeps. Lastly, our iPSC model was compared to spheroids consisting of freshly isolated primary human liver cells. Gene expression levels of cell type-specific markers such as CYP3A4 and PDGFRβ at day 7 of primary liver spheroid cultures were similar to day 13 of iPSC liver cultures, suggesting that the iPSC-derived liver spheroid culture is an adequate in vitro human liver model. Comparisons of cytotoxicity in iPSC versus primary human liver spheroids, induced by APAP and other compounds are still ongoing.
Conclusions: We have established a robust human iPSC-derived liver culture model that can be used to mimic fibrosis in vitro as a replacement of primary human 3D models. This model can be used to investigate pathways involved in fibrosis development and to identify new targets for chronic liver disease therapy.

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PREVALENCE OF CHRONIC HEPATITIS E INFECTION IN IMMUNOSUPPRESSED PATIENTS IN BELGIUM.
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Introduction: Hepatitis E virus (HEV) is now considered as the commonest cause of acute viral hepatitis in Western countries, especially genotype 3 and 4. In immunocompromised patients, chronic HEV have been described, leading in certain cases to cirrhosis, decompensation and death or liver transplantation. A recently published study from the UK based on a Markov cohort model suggests that routine screening for HEV in solid organ transplant (SOT) is very likely to be cost-effective, even more in patients with abnormal transaminases. No data are available on the real prevalence and incidence of HEV chronic infections in this population in Belgium.
Aim: To determine the prevalence of HEV in immunosuppressed populations (SOT and patients suffering from hematological diseases) in a single tertiary center in Belgium, Cliniques universitaires Saint-Luc (CUSL), in Brussels.
Methods: From May 2022 to April 2023, we are prospectively screening for HEV all patients transplanted from a liver, kidney, or heart as well as patients suffering from a lymphoma or followed in the hematology department for a bone marrow transplantation and under immunosuppression from at least three months. The screening includes HEV IgG, HEV IgM and PCR with a genotyping if PCR is positive. Collected data include demographic data, past medical history, reason for immunosuppressive therapy and type of immunosuppressive therapy, routine clinical examination and routine blood test including liver function tests.
Results: We present here an intermediate analysis of a 5-months screening. Among 431 patients, 153 were followed for kidney transplantation, 123 for heart transplantation, 110 for liver transplantation and 45 for hematological pathology requiring maintenance immunosuppression. The median age is 60. The sex ratio F/M is 1/2. The IgG HEV seroprevalence is 9,8% in the whole population, 8,7% in kidney transplanted patients, 12,8% in heart transplanted patients, 12,5% in liver transplanted patients and 0% in hematological pathology. A positive HEV PCR was found in 4 patients, all kidney transplanted, and genotype 3c was identified in all of them. They all presented positive HEV IgG and IgM. At the time of the diagnosis, they were all receiving an immunosuppression based on corticosteroids, a calcineurin inhibitor and mycophenolate mofetil. Only one of them had normal liver function tests at diagnosis. For two of them, the HEV PCR remained positive after 3 months and chronic HEV was diagnosed.
Conclusions: The IgG HEV seroprevalence in our population is 9.8%. In the flemisch blood donor population in 2015, the prevalence was 8,71% and in the serum bank between 2006 and 2014, the prevalence was 4,1% et 5,8%, respectively. HEV PCR was only detected in renal transplanted recipients. More data are awaiting to draw conclusions

on the importance of systematic screening in these immunosuppressed populations. This study is supported by a Gilead fellowship grant.

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THE PAN-PPAR AGONIST LANIFIBRANOR IMPROVES INCREASED PORTAL PRESSURE, ENDOTHELIAL DYSFUNCTION AND LIVER HISTOLOGY IN A RAT MODEL OF EARLY NAFLD. S. Chotkoe (1), Y. Liu (2), G. Wettstein (3), J. Junien (3), L. Vonghia (4), H. Ceuleers (2), J. De Man (2), B. De Winter (1), W. Kwanten (4), S. Francque (4) / [1] Universiteit Antwerpen / Antwerp University Hospital, WILRIJK (Antwerpen), Belgium, Laboratory of Experimental Medicine and Paediatrics, [2] University of Antwerp, Antwerp, Belgium, Laboratory of Experimental Medicine and Paediatrics, [3] Inventiva Pharma, Daix, France, Biology and Pharmacology, [4] Universiteit Antwerpen / Antwerp University Hospital, WILRIJK (Antwerpen), Belgium, Gastroenterology & Hepatology.

Introduction: Non-alcoholic fatty liver disease (NAFLD) is currently the most prevalent chronic liver disease worldwide, with no pharmacological therapy licensed yet. We previously demonstrated an increased intrahepatic vascular resistance (IHVR) related to endothelial dysfunction and an altered endothelin-1 (ET-1) pathway in early NAFLD in rats, potentially contributing to disease progression. The pan-peroxisome proliferator-activated receptor (pan-PPAR) agonist Lanifibranor has demonstrated beneficial effects on liver histology in NAFLD patients and was also shown to have favourable effects in preclinical models of cirrhosis and portal hypertension.
Aim: This study aimed to determine the effects of Lanifibranor on the functional intrahepatic vascular alterations and on the histological features in early NAFLD in a preventive set-up in rats.
Methods: Male Wistar rats (n=8 per group) were fed a methionine-choline-deficient diet (MCD) or control diet (CD) for 4 weeks and received simultaneously vehicle or 100 mg/kg Lanifibranor daily via oral gavage QD. In vivo haemodynamics and blood pressures of the carotid artery, portal vein and inferior caval vein were measured, followed by in situ ex vivo liver perfusion in the same animal to assess baseline transhepatic pressure gradient (THPG) at different flows (flow range 10 -50 mL/min). Dose-response curves were subsequently generated with vasoconstrictors ET-1 (dose range 10^-12–3.10^-9 M) and methoxamine (Mx, 10^-6–3.10^-4 M), and vasodilator acetylcholine (ACh, 10^-6–10^-3 M) after Mx preconstriction (at a dose of 3.10^-5 M). Haematoxylin-Eosin (HE) and Sirius red staining was performed on liver tissue for histological examination using the steatosis-activity- fibrosis (SAF) score.
Results: In vehicle-treated animals, livers of MCD rats showed severe, grade 3 steatosis, without inflammation or fibrosis, compared to normal livers in CD rats. Moreover, MCD rats showed a significantly increased portal pressure in vivo compared to CD rats (5.64±0.63 vs. 3.52±0.24 mmHg, $p<0.0001$), and THPG ex vivo was increased as well at every perfusion flow velocity (e.g., at 30 mL/min: 8.78±0.35 vs. 6.73±0.28 mmHg) with $p<0.001$. Furthermore, the MCD rats were significantly hyperreactive to ET-1 and Mx, and hyporeactive to Ach compared to their CD counterparts. Treatment with Lanifibranor induced no changes of in vivo and ex vivo THPG measurements in CD rats, but significantly decreased the portal pressure in vivo (from 5.64±0.63 to 3.08±0.28 mmHg, $p<0.0001$) as well as THPG ex vivo at all flows (e.g., at 30 ml/min: from 8.78±0.35 to 6.31±0.15 mmHg, $p<0.001$) in MCD rats. Lanifibranor improved the hyperreactivity to Mx and tended to improve the hyporeactivity to ACh in MCD rats, although it did not normalise ET-1 hyperreactivity. Histology confirmed that Lanifibranor also improved steatosis in MCD rats compared to vehicle, mainly in the pericentral zones.
Conclusions: The pan-PPAR agonist Lanifibranor substantially reduces the increased portal pressure and related functional intrahepatic vascular alterations associated with early NAFLD. Lanifibranor improves liver histology as well. These data further support the role of intrahepatic vascular alterations in the development in NAFLD and NAFLD-related portal hypertension on the one hand, and the potential of Lanifibranor as treatment for NAFLD on the other hand. A complementary study with the same experimental set-up was performed to unravel the individual contribution of each PPAR isotype (alpha, delta and gamma) to the beneficial effects of Lanifibranor.

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EXCESSIVE PROLIFERATION AFTER EXTENDED HEPATECTOMY COMPROMISES LIVER FUNCTION IN MICE. M. De Rudder (1), I. Leclercq (2), A. Dili (3) / [1] Université Catholique de Louvain (UCLouvain), Brussels, Belgium, Laboratory of Hepato-Gastroenterology, [2] Université Catholique de Louvain (UCLouvain), Brussels, Belgium, Laboratory of Hepato-gastroenterology, [3] Centre Hospitalier Universitaire Mont-Godinne, Belgium, HPB surgery unit.

Introduction: Small for size syndrome (SFSS) is a post- extended hepatectomy liver failure. Hepatocyte hyperproliferation and vascular damage are thought to be the two major culprits leading to organ failure. In previous studies from the lab, we showed that exposure to hypoxia prevented mortality in mice after an extended hepatectomy. Higher survival rates were associated with an early trigger of endothelial cell proliferation and subsequently a denser sinusoidal network with larger sinusoids, rescue of the disturbed perfusion found after a SFSS-setting hepatectomy, and recruitment of endothelial progenitor cells. Surprisingly, decreased hepatocyte proliferation was associated with survival. Here we