MODELLING ISCHEMIC CHOLANGIOPATHY: EXPLORING PRIMARY HUMAN CHOLANGIOCYTE ORGANOIDS AS A TRANSLATIONAL RESEARCH TOOL. E. Dos Santos (1), M. Meuwis (2), A. De Roover (3), O. Detry (3), N. Gilbo (3) / [1] Université de Liège, Liège, Belgium, CREDEC, [2] ULiège, Belgium, Hepato-Gastroenterology and Digestive Oncology Department, [3] ULg and CHU de Liège, Liège, Belgium, sciences cliniques.

Introduction: Liver transplantation is the best treatment for end-stage liver failure and primary liver cancer. Although long-term results are excellent, one-third of the recipients will develop ischemic cholangiopathy (IC) within the 1st year post-transplant. IC causes diffuse biliary strictures resulting in sepsis, secondary biliary cirrhosis, and graft failure, with 50% of IC patients requiring re-transplantation. Ischemic injuries occurring at the time of organ procurement and preservation are the main risk factors for IC. However, the limited understanding of cholangiocyte ischemia-reperfusion injury is a significant barrier to preventing and treating IC. Deceased organ donation occurs through two main pathways: donation after brain death (DBD) and donation after circulatory death (DCD). In both donor types, the liver graft is preserved with static cold storage in hypothermic, ischemic conditions. DCD donors don't meet the criteria for brain death but have no viable treatment options. In such cases, life-supporting can be withdrawn for the purpose of organ donation, resulting in circulatory arrest and ensuing brain death. Therefore, DCD livers suffer more severe injuries due to the additional normothermic ischemia during circulatory arrest. Consequently, DCD liver recipients face 11 times higher risk of developing IC.
Aim: The main objective of this work was to establish human cholangiocyte organoids (CO) as a preclinical model to investigate the pathophysiology of cholangiocyte injury during liver preservation and transplantation. Additionally, we pilot the comparison of CO generated from DBD and DCD donors to investigate if donation-induced injury traits are retained in this model. Organoid development and characterisation are detailed using RT-PCR and imaging techniques.
Methods: The generation of human CO started with the procurement of gallbladders from both DBD (n=2) and DCD (n=2) donor livers accepted for transplantation. The gallbladder mucosa was then subjected to mechanical dissociation. Cholangiocytes were isolated and cultured in matrigel with R-spondin, Epithelial Growth Factor, Dickkopf-related protein 1, and Rho kinase inhibitor Y-27632. The generation of CO from primary human cholangiocytes was confirmed via RT-qPCR through the expression of the specific cholangiocellular markers: cytokeratin 7 (KRT7) and 19 (KRT19) and gamma-glutamyl transferase 1 (GGT1), and the stemness marker SRY-Box Transcription Factor 9 (SOX9). CO development was monitored daily for a month at passages 0, 1, and 3 using optical and live cell imaging (Incucyte). We manually counted organoid numbers and measured their diameter using the Incucyte, comparing CO derived from DBD and DCD samples with 2-way ANOVA. A p-value<0.05 was considered significant.
Results: CO were visible in bright field optical imaging starting from the first culturing day as small hollow spheres lined with cells surrounding a clear lumen. In the following days, all CO progressively increased their size and showed signs of division and propagation, reaching confluence within an average of 6 (+-1) days. Successful generation of CO was confirmed by the positive expression of the mature cholangiocyte-specific marker genes KRT7, KRT19, GGT1, and SOX9 by RT-qPCR, regardless of the type of donation. Additionally, live cell imaging showed that primary cholangiocytes procured from DCD donors generated significantly fewer CO than cholangiocytes derived from DBD donors ($p=0.001$) at passage 0. Although, in both donation groups the number of organoids increased significantly over time ($p<0.0001$), the CO generated from DCD samples grew at a significantly slower rate compared to CO generated from DBD donors ($p<0.0001$). This difference was not visible at passage 1 and 3. There was no difference in CO size between donor groups at any of the considered culture passages. Further phenotypic and functional characterization is ongoing.
Conclusions: Our results indicated that human gallbladders procured from liver grafts accepted for transplantation are an adequate source of primary cholangiocyte for the successful generation of CO retaining both stemness and mature cholangiocyte profiles. Furthermore, our preliminary results suggest that this CO model can retain donation-induced injury traits, such as lower cholangiocyte viability and defective regeneration typically observed in DCD livers, at least at passage 0. Nevertheless, further expansion of the sample size and thorough characterization and functional testing are necessary to validate this model and will be the object of further experimentations.

DRAGON PLC: AN INTERNATIONAL MULTICENTRE RANDOMIZED CONTROLLED TRIAL TO COMPARE COMBINED PORTAL AND HEPATIC VEIN EMBOLIZATION WITH PORTAL VEIN EMBOLIZATION ALONE IN PATIENTS WITH PRIMARY LIVER CANCERS. S. James (1), J. Smits (1), R. Korenblik (1), M. Dewulf (1), C. van der Leij (2), O. Detry (3), R. van Dam (1), *. The DRAGON Trials Collaborative (4) / [1] Maastricht University Medical Centre, Maastricht, The Netherlands, Surgery, [2] Maastricht University Medical Centre, Maastricht, The Netherlands, Radiology and Nuclear Medicine, [3] CHU de Liège, Liège, Belgium, Abdominal Surgery and Transplantation, [4]

Maastricht University Medical Centre, Maastricht, The Netherlands, Surgery and Interventional Radiology.
Introduction: Patients with primary liver cancers (PLC) are often ineligible for liver resection because an inadequate future liver remnant (FLR) poses too high a risk of post-hepatectomy liver failure. FLR growth can be induced by portal vein embolization (PVE), the current standard. PVE combined with embolization of the hepatic veins (PVE/HVE) is expected to increase resectability, degree of hypertrophy and kinetic growth rate of the FLR.
Aim: The aim of the DRAGON PLC RCT is to evaluate the superiority of PVE/HVE over PVE regarding overall survival and resectability in patients with PLC.
Methods: During 2.5 years, 358 patients with perihilar cholangiocarcinoma (pCCC), intrahepatic cholangiocarcinoma (iCCC) or hepatocellular carcinoma (HCC) requiring preoperative FLR regeneration will be randomized to PVE/HVE or PVE (1:1, stratified by tumour type). Work instructions standardize the interventions. Clinical and imaging data are collected 1, 3 and 6 weeks after PVE(/HVE), concerning resection, and during 5 years of follow-up. Split primary endpoints are defined as: FLR considered sufficient for resection 3 weeks after embolization and 5-year overall survival. FLR volume and function increase, quality of life and costs, among other secondary endpoints, are also assessed.
Results: The DRAGON PLC RCT has been granted funding by the Dutch Cancer Society, ZonMw and KCE, and is due to start accrual at the beginning of 2025. Nine Belgian centres will participate in the trial, with the CHU de Liège as the Belgian coordinating centre.
Conclusions: The effect of PVE/HVE on resectability and survival is expected to illuminate new treatment pathways, ensuring rapid, safe and cost-effective extended liver resections.

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CHARACTERIZATION OF IMMUNE CELL POPULATIONS IN THE MICROENVIRONMENT OF MICE LIVER DURING THE PROGRESSION OF LIVER FIBROSIS TO HEPATOCELLULAR CARCINOMA.

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Introduction: Hepatocellular carcinoma (HCC) is a classic inflammation-driven cancer, with the majority of cases arising in the setting of chronic liver disease (CLD), especially cirrhosis. Profiling the dynamic changes in immune cell populations during the progression of CLD from fibrosis to HCC is critical for enhancing prognostic accuracy and improving therapeutic strategies.
Aim: This study aims to characterize the dysregulated immune microenvironment and spatial distribution of immune cells as CLD progresses from fibrosis to HCC using diethylnitrosamine (DEN) and carbon tetrachloride (CCl4) induced mouse model.
Methods: A single injection of DEN at 4 weeks of age, followed by continuous injections of CCl4 for 6 and 21 weeks were administered respectively, to induce liver fibrosis and HCC in mice. Immune cell profiling was performed using multiplex immunofluorescence on formalin-fixed paraffin-embedded (FFPE) liver sections from control, fibrosis, and HCC groups. The HCC group was further subdivided into tumour, invasive margin (IM), and non-tumour tissue (NTT) regions, to study in detail the tumour microenvironment and its interaction with the surrounding tissues.
Results: Immune profiling revealed significant changes in hepatic immune cell populations across the disease stages. Total leukocyte and myeloid cell densities (cells/mm2) remained largely consistent across groups. However, macrophage populations exhibited significant variation. Kupffer cell (KC) levels were stable, while infiltrating macrophages (Inf mph) increased markedly, rising approximately 5-fold in tumour regions and 10-fold in the IM and NTT regions of HCC livers and 3-fold in fibrosis livers, compared to controls. T lymphocyte (CD3+) populations showed a progressive increase, particularly CD4+ T cells, which exhibited a 5-fold increase in fibrosis and a 10-fold increase in the IM and NTT regions of HCC livers compared to controls. Among the different immune cell populations analysed, regulatory T cells (Tregs) demonstrated the most substantial rise, increasing 125-fold in the IM region and 80-fold in the NTT regions of HCC livers. In the HCC liver microenvironment, Spearman's correlation analysis revealed complex interactions between various immune cell populations. Notable correlations in the tumour region included those between proliferating cells and proliferating KCs ($r = 0.59$, $p = 0.035$), proliferating hepatocytes and proliferating infiltrating macrophages ($r = 0.67$, $p = 0.012$), tumour CD8+ T cells and proliferating infiltrating macrophages ($r = 0.60$, $p = 0.032$), and KCs and Tregs ($r = -0.56$, $p = 0.048$). Similarly, in the IM region, significant correlations were observed between KCs and proliferating hepatocytes ($r = 0.72$, $p = 0.0096$), CD8+ T cells and proliferating infiltrating macrophages ($r = 0.73$, $p = 0.009$), tumour CD4+ T cells and IM CD4+ T cells ($r = 0.60$, $p = 0.030$), and tumour CD8+ T cells and IM Tregs ($r = -0.63$, $p = 0.023$).
Conclusions: This study provides a comprehensive analysis of immune cell dynamics during CLD progression, highlighting significant immune cell infiltration and redistribution, particularly in IM region of the HCC liver. The marked increase in Inf mph and Tregs suggests a skewed immunosuppressive phenotype and evolving immune microenvironment as fibrosis progresses towards HCC.