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 In such cases, life-supporting can be withdrawn for the purpose of organ donation, resulting in circulatory arrest and ensuing brain death.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]