

# Liver transplantation in patients with hilar cholangiocarcinoma : single center experience

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Dinjar Kujundžić, Petra; Ledinski, Ivan; Ostojić, Ana; Sobočan, Nikola; Gašparov, Slavko; Kocman, Branislav; Jadrijević, Stipislav; Mikulić, Danko; Filipec Kanižaj, Tajana

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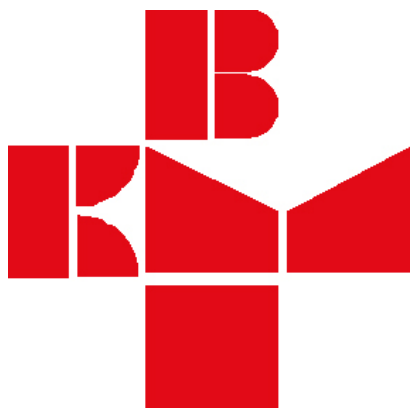
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# PROGRAMME & ABSTRACTS

## CHOLANGIOCYTES IN HEALTH AND DISEASE: FROM BASIC SCIENCE TO NOVEL TREATMENTS

09-11 JUNE 2017  
OSLO, NORWAY

**Scientific Organising Committee**

Jesus M. Banales, *Spain*

Peter L. Jansen, *The Netherlands*

Nicholas F. LaRusso, *United States*

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# WELCOME MESSAGE

Dear Colleagues,

As organizing committee, we welcome you to this **EASL monothematic conference on ‘Cholangiocytes in Health and Disease: From Basic Science to Novel Treatments’**.

Bioengineers and physicists are linking up with biologists, translational scientists and clinicians to get a better understanding of the complexity of the many acquired and genetic cholestatic liver diseases. Novel genetic information, multimodal imaging, pathway analysis, digitalized microscopy and reconstructions and novel mouse models shed a new light on the relation between anatomy and function in the liver in general and in the biliary tree in particular. Intravital 2-photon microscopy makes us aware of the dynamics and function of cholangiocytes, bile canaliculi and bile ducts. Metabolomic analysis point to hitherto hidden spatio-temporal relations and disturbances that play an underestimated role in the developmental phases of cholestatic liver disease.

The discovery of nuclear hormone receptors and signaling paths, involved in the regulation of gene expression, give rise to new drugs that suppress disease activity and in the long run may be able to cure these diseases. An interesting side effect of these developments is the awareness among researchers and translational scientists that there are remarkable parallels between cholestatic and metabolic liver disease. Many new drugs, in trial for traditional cholestatic liver diseases, are also under investigation for the treatment of non-alcoholic steatohepatitis. These diseases may have more in common than traditional teaching tells us.

Cholangiocytes not only have a barrier function, separating toxic bile from cells and structures in and around the bile ducts, but these cells are metabolically highly active and secrete copious amounts of water, electrolytes and bicarbonate. In addition, bile ducts harbor stem cells are important for restoration and regeneration of the entire liver. Bile duct proliferation may play a yet incompletely understood role in epithelial mesenchymal transition and liver fibrosis. Disturbances of any of these structures and functions may lead to disease manifestations and may underlie cholestatic liver disease.

Immunology and metabolism for long have been antipodes in the cause-and-effect discussion. Now it becomes more and more clear that immunological and toxic injury are closely related and amplify each other. New thinking is required that covers both fields. Although cholangiocytes reside deep in the liver, functionally these cells are at the cross roads between environment and self. Bacterial products from the gut are absorbed,

secreted in bile where they affect cholangiocytes. Recent discoveries have led to insights and a better understanding of the close interaction between cholangiocytes and gut.

Hepatology for a long time has been very much hepatocyte-oriented. Time has come to shift gears and focus on liver cells that, more than hepatocytes, have roles to play in inflammation, fibrosis, proliferation and regeneration.

We believe that going to a conference is part of one's scientific life. You not only go there to listen to top-researchers and get new ideas but also to contribute and interact. We hope that during this conference we create an atmosphere that leads to lasting interactions above all to new and exciting science.

## SCIENTIFIC ORGANISING COMMITTEE

**Dr Jesus Banales**

*Spain*



**Prof. Peter Jansen**

*The Netherlands*



**Prof. Nicholas LaRusso**

*United States*



**Dr Marco Marzioni**

*Italy*



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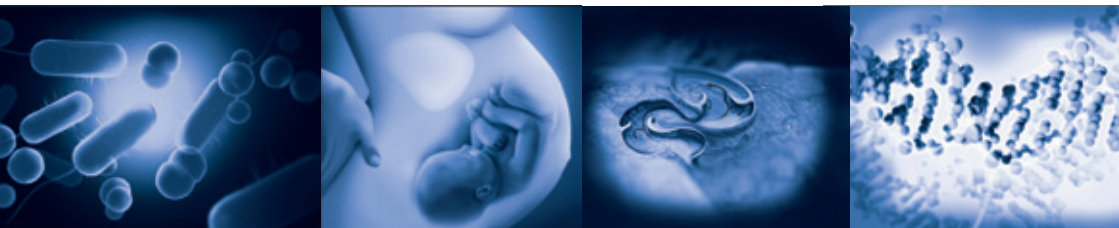
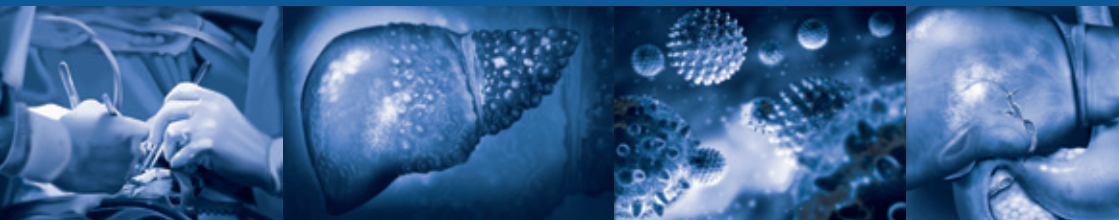
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# GENERAL INFORMATION

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# GENERAL INFORMATION

## CONFERENCE VENUE

Radisson Blu Plaza Hotel, Oslo  
 Sonja Henies plass 3  
 P.O. Box 9206 N-0185  
 Oslo, Norway

## DISCOVER OSLO

City website:

<http://www.visitoslo.com>

Oslo is one of Europe's fastest-growing cities, with a population approaching 700,000 and new neighbourhoods with eye-catching architecture are popping up.

The largest city in Norway is quickly transforming into a cosmopolitan hub with an abundance of world-class museums, restaurants and art, but still maintains the relaxed atmosphere of a much smaller town.

The city is nestled between the Oslofjord and hundreds of square miles of forested hills, and is a green city in more ways than one. The compact city centre is easily explored on foot or by bike, and an efficient public transport system makes the whole city accessible without a car.

Norway's capital since 1814, Oslo is home to the Norwegian government and the Royal Family.

The country's largest cultural institutions, which include the Norwegian Opera & Ballet, the National Theatre and the

National Museum of Art, Architecture and Design, present first-rate art exhibitions and opera, ballet and theatre performances.

Live music is a big part of the city's identity, and every year Oslo's clubs and arenas host thousands of concerts that showcase the talents of everyone from local bands to international superstars. Several large pop and rock festivals are held every summer, and there are annual festivals for genres ranging from chamber music to heavy metal.

## CLIMATE

Thanks to the Gulf Stream bringing temperate water from the Gulf of Mexico, Oslo's climate is milder than what its latitude would imply.

Average temperatures in June:

15,2 to 16,4°C

Min. temperature: 6,1°C

Max. temperature: 30,5°C

## LANGUAGE

The official language of the conference is English.

## NAME BADGES

All participants are kindly requested to wear their name badges throughout the EASL Monothematic Conference in order to be admitted to the lecture halls and other scheduled activities.

## REGISTRATION AND ACCOMMODATION

All participants are invited to register online in order to save time upon their arrival at the conference.

Hotel accommodation for the EASL Monothematic Conference will be offered to participants during the online registration process. Detailed information, as well as access to the online registration is available on the website. Registered participants are entitled to reduced rates in the conference hotel.

## REGISTRATION DESK

The onsite registration desk at the conference venue will be open at the following times:

Friday	
9 June 2017	from 12:00 to 19:00
Saturday	
10 June 2017	from 08:00 to 19:00
Sunday	
11 June 2017	from 08:00 to 10:30

## CME ACCREDITATION

The “EASL Monothematic conference – Oslo, Norway, 09-11 June 2017” is accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide the following CME activity for medical specialists. The EACCME is an institution of the European Union of Medical Specialists (UEMS), [www.uems.net](http://www.uems.net).

The EASL Monothematic conference – Oslo, Norway, 09-11 June 2017’ is designated for a maximum of (or ‘for up to’) 12 hours of European external CME credits. Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity.

Through an agreement between the European Union of Medical Specialists and the American Medical Association, physicians may convert EACCME credits to an equivalent number of AMA PRA Category 1 Credits™. Information on the process to convert EACCME credit to AMA credit can be found at [www.ama-assn.org/go/internationalcme](http://www.ama-assn.org/go/internationalcme).

Live educational activities, occurring outside of Canada, recognized by the UEMS-EACCME for ECMEC credits are deemed to be Accredited Group Learning Activities (Section 1) as defined by the Maintenance of Certification Program of The Royal College of Physicians and Surgeons of Canada.

## EACCME CREDITS

Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity. The EACCME credit system is based on 1 ECMEC per hour with a maximum of 3 ECMECs for half a day and 6 ECMECs for a full-day event.



## TRANSPORT TO THE VENUE

The conference venue, the Radisson Blu Plaza Hotel Oslo, is 30 minutes away by direct train (50 minutes by car/taxi) from Oslo Airport (OSL).

### By train

Exit the airport and walk 6 minutes towards the Oslo Lufthavn train station. Take the R10 train (23 minute ride, 2 stops). Exit the train at Oslo Central Station (Oslo S). Walk 2 minutes to reach the Radisson Blu Plaza Hotel Oslo. The ticket costs NOK 92.00.

### By car

Head north on Lufthavnvegen. Drive from E6 to Nordstrand, Oslo. Take exit 3 from E6. Follow Rv162 to Sonja Henies plass in Grünerløkka.

## PARTICIPANTS' LIST

The participants' list will be displayed at the conference.

## DRESS CODE AND SMOKING POLICY

Dress code is informal for all occasions. This will be a non-smoking event.

## BANKING, SAFETY AND SECURITY

The currency used in Norway is the Norwegian Krone (NOK). Foreign currency can be exchanged at banks, bureau de change and automatic currency exchange machines.

Please do not leave bags or suitcases unattended at any time, whether inside or outside the session halls. Hotels strongly recommend that you use their safety deposit boxes for your valuables.

## LIABILITY AND INSURANCE

The EASL Office cannot accept liability for personal accidents or loss of or damage to private property of participants. Participants are advised to take out their own personal travel and health insurance for their trip.

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30 SEPT - 30 NOV**

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BETTER FUNDING**



# PhD

## STUDENTSHIP

### JUAN RODES

APPLICATION PERIOD:  
**30 SEPT - 30 NOV**

# SCIENTIFIC PROGRAMME

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## DAY I – FRIDAY 9 JUNE 2017

13:30 – 14:00 *Welcome and introductory remarks*

**Jesus M. Banales**, *Spain*

**Peter Jansen**, *The Netherlands*

**Nicholas F LaRusso**, *United States*

**Marco Marzioni**, *Italy*

14:00 – 14:30 **STATE-OF-THE-ART LECTURE**

*Cholangiocyte pathobiology: Past, present and future*

**Nicholas F LaRusso**, *United States*

### I. THE BILIARY TREE

**Chairs:** **Gianfranco Alpini**, *United States*

**Joost Drenth**, *The Netherlands*

14:30 – 14:55 **3D Anatomy of the biliary tree: Intravital two-photon based functional imaging of bile salt transport in cholestasis – pathophysiology of bile infarct formation**

**Jan Hengstler**, *Germany*

14:55 – 15:20 **Development of the biliary tree**

**Frederic Lemaigre**, *Belgium*

15:20 – 15:45 **Liver injury combined with inhibition of hepatocyte proliferation leads ductular cells to function as facultative stem cells for hepatocytes**

**Stuart Forbes**, *United Kingdom*

15:45 – 16:10 **Repopulating the biliary tree from the peribiliary glands**

**Robert Porte**, *The Netherlands*

16:10 – 16:35 **Bio-engineering the biliary tree**

**Fortis Sampaziotis**, *United Kingdom*

16:35 – 16:50 *Coffee break*

## 2. CHOLANGIOCYTE PATHOPHYSIOLOGY

**Chairs: Marco Marzioni, *Italy***  
**Michael Trauner, *Austria***

- 
- 16:50 – 17:15 **Animal models of biliary injury and altered bile acid metabolism**  
**Luca Fabris, *Italy***
- 
- 17:15 – 17:40 **Mechanisms of regulation of cholangiocyte response to injury**  
**Gianfranco Alpini, *United States***
- 
- 17:40 – 18:05 **Non coding-RNAs and extracellular vesicles**  
**Jesus M. Banales, *Spain***
- 
- 18:05 – 18:30 **Genetics and mechanisms of biliary cystogenesis: Impact in the clinical practice**  
**Joost Drenth, *The Netherlands***
- 
- 18:30 – 18:55 **Pathogenesis and 3D structure of biliary fibrosis**  
**Massimo Pinzani, *United Kingdom***
- 

19:00 – 19:30 *ePoster session 2 and cocktail reception*

19:30 – 20:00 *ePoster session 3 and cocktail reception*

### 3. BILE ACIDS AND THE BILIARY TREE: RECEPTORS AND BIOLOGICAL EFFECTS

**Chairs: Jose J. G. Marin, *Spain***  
**Gustav Paumgartner, *Austria***

08:30 – 08:55 **Nuclear hormone receptors as drug targets**  
**Bart Staels, *France***

08:55 – 09:20 **Gut and liver interactions**  
**Antonio Moschetta, *Italy***

09:20 – 09:45 **TGR5 in physiology and disease**  
**Verena Keitel, *Germany***

09:45 – 10:10 **Bile acids, FGF15/19 and liver regeneration:  
from mechanisms to clinical applications**  
**Matias Avila, *Spain***

10:10 – 10:35 *ePoster session 4 and coffee break*

10:35 – 11:00 *ePoster session 5 and coffee break*

### 4. CLINICAL CONSEQUENCES OF ALTERED BILE ACID METABOLISM

**Chairs: Nicholas F. LaRusso, *United States***  
**Peter Jansen, *The Netherlands***

11:00 – 11:25 **The molecular mechanism of cholestatic itch**  
**Ronald Oude Elferink, *The Netherlands***

11:25 – 11:50 **Heart and bile acids**  
**Catherine Williamson, *United Kingdom***

11:50 – 12:15 **Bile acid-induced nephropathy**  
**Peter Fickert, *Austria***

12:15 – 13:45 *Lunch and poster viewing*

12:45 – 13:15 *ePoster session 6*

13:15 – 13:45 *ePoster session 7*

## 5. ABSTRACT PRESENTATIONS FROM YOUNG INVESTIGATORS

**Chairs: Jesus M Banales, *Spain***  
**Ronald Oude Elferink, *The Netherlands***

13:45 – 14:00 **Serum extracellular vesicles contain protein biomarkers for primary sclerosing cholangitis and cholangiocarcinoma**  
**Ander Arbelaz, *Spain***

14:00 – 14:15 **The role of ageing in the pathophysiology of the biliary epithelium: identification of a molecular profile**  
**Luca Maroni, *Italy***

14:15 – 14:30 **Inhibition of Src tyrosine kinase restores CFTR function in cystic fibrosis cholangiocytes derived from human induced pluripotent stem cells (iPSC) and improves the response to CFTR potentiators and correctors used in therapy**  
**Romina Fiorotto, *United States***

14:30 – 14:45 **Chronic treatment with clinic dose of metformin completely reverses the epithelial to mesenchymal transition (EMT) in human intrahepatic cholangiocarcinomas (iCCAs) by activated AMPK / Foxo3a pathway**  
**Sabina Di Matteo, *Italy***

14:45 – 15:00 **The bile acid receptor TGR5 regulates paracellular permeability and protects the liver through an impact on the tight junction protein JAM-A**  
**Grégory Merlen, *France***

## 6. IMMUNITY IN THE BILIARY TREE

**Chairs: Jesus Prieto, *Spain***  
**Erik Schruppf, *Norway***

15:00 – 15:25 **Mucosal immunity of the biliary tree in normality and disease**  
**David Adams, *United Kingdom***



15:25 – 15:50 **How the biliary tree maintains immune tolerance?**  
**Xiong Ma, China**

15:50 – 16:15 **Bile acid homeostasis and immunity  
of the liver**  
**Christoph Schramm, Germany**

16:15 – 16:40 **IgG4 cholangiopathy, a new entity**  
**Ulrich Beuers, The Netherlands**

16:40 – 17:10 *ePoster session 8 and coffee break*

## 7. BILIARY CARCINOGENESIS

**Chairs: Domenico Alvaro, Italy**  
**Matias Avila, Spain**

17:10 – 17:35 **Target molecules in cholangiocarcinoma cells**  
**Gregory Gores, United States**

17:35 – 18:00 **Stem cell origin of cholangiocarcinoma**  
**Tania Roskams, Belgium**

18:00 – 18:25 **Chemoresistance and chemosensitization  
in cholangiocarcinoma**  
**Jose JG Marin, Spain**

18:25 – 18:50 **Tumour stroma in cholangiocarcinoma:  
A novel therapeutic target**  
**Joachim Mertens, Switzerland**

8. MANAGEMENT ISSUES IN BILIARY DISORDERS

**Chairs: Gregory Gores, *United States***

**Raoul Poupon, *France***

- 08:00 – 08:25 **Cholangiopathies in children**  
**Richard Thompson, *United Kingdom***
- 08:25 – 08:50 **Genetics of familial cholangiopathies**  
**Frank Lammert, *Germany***
- 08:50 – 09:15 **Cholangiocarcinoma: Epidemiological trends and clinical management**  
**Shahid Khan, *United Kingdom***
- 09:15 – 09:40 **The endoscopist and malignant and non-malignant biliary obstructions**  
**Stephen Pereira, *United Kingdom***

09:40 – 10:10 *ePoster session 9 and coffee break*

9. ROUNDTABLE: REDIRECTING THE MANAGEMENT OF BILIARY DISORDERS

**Chairs: Ulrich Beuers, *The Netherlands***

**Tom Karlsen, *Norway***

- 10:10 – 10:25 **Biomarkers for cholangiopathies and cholangiocarcinoma**  
**Daniel Gotthardt, *Germany***
- 10:25 – 10:40 **Assessment of hepatic secretion function with 11C-cholylsarcosine PET/CT**  
**Michael Sorenson, *Denmark***
- 10:40 – 10:55 **The future of therapy for cholangiopathies**  
**Michael Trauner, *Austria***
- 10:55 – 11:10 **Cholestatic drug induced liver injury**  
**Raul Andrade, *Spain***

- 11:10 – 11:25 **Cell transplantation**  
**Domenico Alvaro, Italy**
- 
- 11:25 – 11:40 **Endpoints in the designing clinical trials**  
**Cyriel Ponsioen, The Netherlands**
- 
- 11:40 – 11:55 **Principles of value based medicine as applied to cholangiopathies**  
**Mario Strazzabosco, Italy**
- 

- 11:55 – 12:45 *Panel discussion*
- 12:45 – 13:00 *Concluding Remarks*
- 13:00 – 14:00 *Farewell Lunch*



**ePOSTER  
PRESENTATION  
SCHEDULE**

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## DAY I – FRIDAY 9 JUNE 2017

ePoster session I – Presentations of the ePoster session I have been rescheduled in other ePoster sessions

ePoster session 2 – 19:00 – 19:30

Screen	Title	Abstract	Presenting author
1	Combined targeting of cholangiocytes and activated stromal fibroblasts with a Bcl-xL inhibitor ameliorates liver fibrosis in Mdr2 <sup>-/-</sup> mice	P02.01-YI	<i>Anja Moncsek, Switzerland</i>
2	ATP Binding Cassette B4 (ABCB4) related disease – from genetics to phenotypic variability: a case series	P02.02-YI	<i>Pedro Marques da Costa, Portugal</i>
3	Autotaxin activity predicts transplant-free survival in primary sclerosing cholangitis	P02.03	<i>Johannes R. Hov, Norway</i>
4	Natural Killer T cells are activated in mice with cholestasis	P02.04-YI	<i>Laura Valestrand, Norway</i>
5	Auto-antibodies of the IgG4 and IgG1 subclass against Annexin A11 in IgG4-related disease of the biliary tract and pancreas	P02.05-YI	<i>Lowiek Hubers, The Netherlands</i>
6	Circulating cancer-associated large extracellular vesicles in cholangiocarcinoma	P02.06-YI	<i>Sabine Urban, Germany</i>
7	Cellular senescence exacerbates injury and impairs regeneration in biliary disease	P02.07-YI	<i>Sofia Ferreira-González, United Kingdom</i>

8	CHOP deficiency protects against bile duct ligation-induced cholestatic liver injury by rescuing ER stress-induced loss of intestinal epithelial stemness	P02.08	<i>Runping Liu, United States</i>
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ePoster session 3 – 19:30 – 20:00

Screen	Title	Abstract	Presenting author
1	Role of endoscopic ultrasound with fine needle aspiration for non mass-forming cholangiocarcinomas	P03.01-YI	<i>Andrea Telese, United Kingdom</i>
2	Early identification of candidates for second-line therapies in PBC using a «UDCA-response» model	P03.02	<i>Marco Carbone, United Kingdom</i>
3	microRNA-21 is overexpressed in primary biliary cholangitis and contributes to liver injury and necroptosis in cholestatic bile duct-ligated mice	P03.03-YI	<i>Marta Afonso, Portugal</i>
4	Serial changes in liver stiffness measured by transient elastography and acoustic radiation force impulse imaging in patients with cholestasis	P03.04-YI	<i>Mira Shafik, Egypt</i>
5	Simultaneous deletion of tight junction proteins ZO-1 and ZO-2 in hepatocytes results in the desintegration of the intrahepatic biliary system	P03.05-YI	<i>Noemi Van Hul, Singapore</i>
6	Sensitive detection of cholangiocarcinoma using DNA methylation biomarkers in bile	P03.06	<i>Trine Folseraas, Norway</i>

7	A familial syndrome of primary sclerosing cholangitis with a heterozygous germline mutation in SEMA4D	P03.07	<i>Xiaojun Jiang, Norway</i>
8	NAD <sup>+</sup> /NADH redox status represents an abridged metabolic index that regulates intrinsic apoptosis in human cholangiocytes	P03.08-YI	<i>Jung-Chin Chang, The Netherlands</i>

## DAY 2 – SATURDAY 10 JUNE 2017

ePoster session 4 – 10:10 – 10:35

Screen	Title	Abstract	Presenting author
1	Whole transcriptome analysis of ductular reaction from patients with alcoholic hepatitis. Similarities to ductular reaction in DDC mouse model	P04.01-YI	<i>Beatriz Aguilar, Spain</i>
2	TWEAK/Fn14 signalling drives chemokine secretion in cholangiocarcinoma	P04.02-YI	<i>Benjamin Dwyer, United Kingdom</i>
3	Type I interferons are sensitive inflammatory biomarkers in primary sclerosing cholangitis	P04.03-YI	<i>Christina Mehrfeld, Germany</i>
4	SOX17 selectively sensitizes cholangiocarcinoma cells to anticancer drugs by interfering with promoter activation of the export pumps ABCC3 and ABCG2	P04.04-YI	<i>Elisa Lozano, Spain</i>
5	Alagille syndrome results in loss of polarity of cholangiocytes, rather than cholangiocyte loss per se	P04.05-YI	<i>Emma Andersson, Sweden</i>
6	Protein kinase CK2 as potential therapeutic target in cholangiocarcinoma	P04.06-YI	<i>Giovanni Di Maira, Italy</i>

7	Defects in protein processing may underlie the development of hepatic cysts in patients with polycystic liver disease	P04.07-YI	<i>Liyanne van de Laarschot, The Netherlands</i>
8	The CDE-induced liver injury causes impaired canalicular bile flow and expansion of small biliary branches that connect to intralobular canaliculi to drain bile	P04.08-YI	<i>Laure-Alix Clerbaux, Belgium</i>

## ePoster session 5 – 10:35 – 11:00

Screen	Title	Abstract	Presenting author
1	High-throughput sequencing analysis of tissue-resident gut and liver B cells reveals antigen-driven clonal expansion in primary sclerosing cholangitis-inflammatory bowel disease	P05.01-YI	<i>Brian Chung, United Kingdom</i>
2	The crucial role of KITENIN in cholangiocarcinogenesis	P05.02-YI	<i>Khac Cuong Bui, Germany</i>
3	Identification and in silico characterization of six novel GANAB mutations in polycystic liver disease	P05.03-YI	<i>Liyanne van de Laarschot, The Netherlands</i>
4	MiRNA-506 promotes primary biliary cholangitis-like features in cholangiocytes and immune activation	P05.04-YI	<i>Oihane Erice, Spain</i>
5	Role of the ciliopathy protein MKS1 in the homeostasis of bile duct epithelia	P05.05	<i>Pascale Dupuis-Williams, France</i>



6	LY3039478 a notch gamma-secretase inhibitor blocks cholangiocarcinoma growth in a patient-derived xenograft (PDX) model	P05.06-YI	<i>Serena Mancarella, Italy</i>
7	Sox9 is a crucial transcription factor in development of intrahepatic cholangiocarcinoma	P05.07-YI	<i>Xiaodong Yuan, Germany</i>
8	Identification of AGXT as a putative target in the Mdr2 <sup>-/-</sup> murine model of liver cancer	P05.08-YI	<i>Letizia Satriano, Denmark</i>

## ePoster session 6 – 12:45 – 13:15

Screen	Title	Abstract	Presenting author
1	Chronic hepatitis C is associated with increased incidence of gall bladder stones due to enhanced expression of D19H polymorphism in hepatobiliary cholesterol transporter ABCG8	P06.01	<i>Amr Hanafy, Egypt</i>
2	Yap is essential for cholangiocyte homeostasis and its removal leads to loss of ductal integrity and compensatory biliary cell expansion	P06.02-YI	<i>Brian Pepee-Mooney, United States</i>
3	Tridimensional cultures of human biliary tree and hepatic stem/progenitor cells in simulated microgravity perpetuated the stem features and reduced the metabolic oxidation	P06.03-YI	<i>Daniele Costantini, Italy</i>
4	PGC-1 $\beta$ promotes development of hepatocellular carcinoma limiting ROS detrimental accumulation	P06.04-YI	<i>Elena Piccinin, Italy</i>
5	Characterization of the mitochondrial phenotype in primary biliary cholangitis patients' cholangiocytes	P06.05-YI	<i>Filip Włysokinski, Canada</i>

6	Differential effects of FXR or TGR5 activation in cholangiocarcinoma progression	P06.06-YI	<i>Ibone Labiano, Spain</i>
7	Relationship of IMP3 and HER3 expression with TH17 cells in cholangiocarcinomas	P06.07-YI	<i>Yulian Ananiev, Bulgaria</i>
8	Risk reduction with obeticholic acid treatment in patients with primary biliary cholangitis not achieving the POISE primary endpoint	P06.08-YI	<i>Maren Harms, The Netherlands</i>

ePoster session 7 – 13:15 – 13:45

Screen	Title	Abstract	Presenting author
1	3D-reconstruction of the murine biliary tree during cholestasis using two different techniques: corrosion casts and Microfil®-MV. Methods` descriptions and first results	P07.01	<i>Beate Richter, Germany</i>
2	Colesevelam attenuates cholestatic liver and bile duct injury in Mdr2 <sup>-/-</sup> mice by modulating composition, signaling and excretion of fecal bile acids	P07.02	<i>Michael Trauner, Austria</i>
3	S-Adenosyl L-Methionine-induced protein S-glutathionylation may modulate immune responses in patients with Primary Biliary Cholangitis	P07.03	<i>Ewa Kılanczyk, Poland</i>
4	Precision-cut bile duct slices as a model to study regeneration of bile ducts of human donor livers after ischemic preservation injury	P07.04-YI	<i>Iris De Jong, The Netherlands</i>

5	Autoimmune hepatitis: Evaluation of specificity of various histological features and development of a modified scoring system	P07.05	<i>Joanna Gibson, United States</i>
6	Splanchnic release contributes to the elevated pool of FGF19 in the circulation of patients with obstructive cholestasis	P07.06-YI	<i>Kiran Koelfat, The Netherlands</i>
7	Hypothermic oxygenated machine perfusion reduces biliary reperfusion injury after transplantation of donation after circulatory death livers	P07.07-YI	<i>Rianne Van Rijn, The Netherlands</i>
8	Functional role of extracellular signal-regulated kinase 5 on cholangiocarcinoma cells	P07.08	<i>Alessandra Gentilini, Italy</i>

## ePoster session 8 – 16:40 – 17:10

Screen	Title	Abstract	Presenting author
1	Identification of tumour suppressive and oncogenic microRNAs in gallbladder carcinoma	P08.01	<i>Benjamin Goepfert, Germany</i>
2	Biliary microrhamartomas (Von-Meyenburg complexes) are frequently associated with intrahepatic and hilar cholangiocarcinomas in liver	P08.02	<i>Joanna Gibson, United States</i>
3	The positive effect of exosomes on epithelial-mesenchymal transition through hedgehog signalling in cholangiocarcinoma cells	P08.03	<i>Ozlem Kucukoglu, Germany</i>
4	The autophagy marker predicts tumor recurrence in intrahepatic cholangiocarcinoma patients after surgical resection	P08.04	<i>Chin-Wen Lin, Taiwan</i>

5	Liver transplantation in patients with hilar cholangiocarcinoma- single center experience	P08.05-YI	<i>Petra Dinjar Kujundžić, Croatia</i>
6	Liver progenitor cells significantly contribute to hepatocyte pool in chronic liver injury and cirrhosis: a kinetic study in mice	P08.06-YI	<i>Rita Manco, Belgium</i>
7	Preliminary results from application of image processing tools and quantitative analysis of MRCP datasets of various aetiologies	P08.07	<i>Andy McKay, United Kingdom</i>
8	Common variant p.D19H of the hepatobiliary sterol transporter ABCG8 is associated with increased gallstone risk and distorted sterol homeostasis in children	P08.08-YI	<i>Marcin Kravczyk, Poland</i>

## DAY 3 – SUNDAY 11 JUNE 2017

ePoster session 9 – 09:40 – 10:10

Screen	Title	Abstract	Presenting author
1	The anti-inflammatory receptor TREM-2 protects the liver from cholestatic injury in mice	P09.01-YI	<i>Aitor Esparza-Baquer, Spain</i>
2	CD86+/CD206+ tumor-associated macrophages predict prognosis of patients with intrahepatic cholangiocarcinoma	P09.02-YI	<i>Dalong Sun, China</i>
3	Fluoroscopically guided biopsies do not seem to improve cancer detection rates in patients with biliary strictures undergoing brush cytology during ERCP	P09.03-YI	<i>Daniela Reis, Portugal</i>

4	Hepatoprotective effect of S-Adenosyl L-Methionine (SAME) in oxidative stress: in vitro study on hepatocytes and cholangiocytes	P09.04	<i>Ewa Kilanczyk, Poland</i>
5	Anti-apoptotic role of c-FLIP in human cholangiocarcinoma: activation of Fas/FasL pathway	P09.05-YI	<i>Gianluca Carnevale, Italy</i>
6	Insulin-like growth factor signaling axis confers acquired resistance to EGFR targeting drug erlotinib by inducing EMT/CSC in human cholangiocarcinoma cells	P09.06	<i>Laura Fouassier, France</i>
7	Epigenetic profiling of premalignant and malignant stages of cholangiocarcinoma	P09.07-YI	<i>Ursula Lemberger, Austria</i>

# INVITED SPEAKERS' ABSTRACTS

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# State-of-the-art lecture: Cholangiocyte pathobiology: Past, present and future

**Nicholas LaRusso**\*<sup>1</sup>

<sup>1</sup>*Mayo Clinic, Rochester, MN, United States*

**Corresponding author's email:** [larusso.nicholas@mayo.edu](mailto:larusso.nicholas@mayo.edu)

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Over the last several decades, the research community has increasingly recognized the importance of cholangiocytes, the epithelial cells that line the biliary tree, in both normal physiology and in disease. Support for this conclusion is based on a variety of different metrics, including the number of publications, the attention given to cholangiocyte pathobiology by national and international societies, and the number of scientists involved in cholangiocyte research. The factors that have contributed to this increasing interest are the subject of this presentation. They include: 1) the development of technologies and experimental models to study cholangiocytes; 2) the characterization of the transporters, exchangers, and channels and their topographical distribution on cholangiocytes; and 3) the concept that a group of diseases, called the cholangiopathies, while different in etiologies, natural histories and clinical outcomes, share the common feature of involvement of cholangiocytes in their pathogenesis. Given their clinical importance, particular attention has been paid to the following categories of cholangiopathies; immune-mediated, infectious, genetic, idiopathic, and neoplastic. In spite of significant insights into the pathogenetic mechanisms of these cholangiopathies, therapies which significantly impact the natural history of these diseases are still very limited. It is likely that future efforts in cholangiocyte biology will continue to include: 1) establishing new techniques and models; 2) the use of unbiased, multiomic approaches prior to hypothesis-driven experiments; 3) further efforts to understand normal cholangiocyte biology as a prelude to hypothesis-driven experiments to understand cholangiocyte pathobiology; 4) being attentive to new and important areas developing in science in general (e.g., cell-cell communication, the microbiome, processes such as senescence and autophagy shown to be critically important in other diseases, the exposome, etc.); and 5) continued international conversations and collaborations that will help determine the pace of advancement.

# 3D Anatomy of the biliary tree: Intravital two-photon based functional imaging of bile salt transport in cholestasis – pathophysiology of bile infarct formation

Ahmed Ghallab<sup>1</sup>, Nachiket Vartak<sup>1</sup>, Peter L. Jansen<sup>2</sup>, Georgia Günther<sup>1</sup>, Jan G. Hengstler<sup>\*1</sup>, Raymond Reif<sup>†</sup>

<sup>1</sup>Leibniz Research Centre for Working Environment and Human Factors at the Technical University Dortmund, Dortmund, Germany, <sup>2</sup>Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, The Netherlands

**Corresponding author's email:** Hengstler@ifado.de

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The biliary tree can be differentiated into three broad topological domains consisting of the large bile ducts running along the portal veins, the interlobular bile ducts that form a mesh around the portal veins, and the canalicular network that borders the hepatocytes (Jansen et al., 2017). These topological domains show different adaptive changes in response to cholestatic liver disease. The large bile ducts respond by enlargement of the ductular diameter through cholangiocyte proliferation. The interlobular bile ducts undergo extensive remodeling, where cholangiocyte proliferation initially causes corrugation of the luminal duct surface, leading to an approximately 5-fold increase in surface area, which is further enhanced by additional duct branching, branch elongation, and loop formation through self-joining (Vartak et al., 2016). The canalicular network shows increased average diameters in cholestasis. These complex responses of the biliary tree in cholestasis seems to serve different purposes - large bile duct expansion maximizes volume, increased interlobular bile duct density maximizes surface and thereby bile resorption capacity, while the functional consequences of alterations of bile canaliculi remain unknown.

To bridge this gap, we introduced two-photon-based imaging that allows to quantify absolute concentrations and the flux of fluorescent bile salts from liver sinusoids via the Disse space and hepatocytes into bile canaliculi in intact livers of living mice. A bolus of choyl-lysyl-fluorescein (CLF) was injected into the tail vein of mice 1, 3, and 21 days after bile duct ligation (BDL) and compared to sham operated mice. A strong uptake block of CLF from sinusoidal blood to hepatocytes was observed at all time periods after BDL, while excretion of CLF into bile canaliculi showed only minor alterations. This basolateral uptake block may be interpreted as a mechanism which protects hepatocytes against increased bile salt concentrations in blood after BDL. This leads to a situation, where the majority of hepatocytes contains only low CLF concentrations. In contrast, a minority of individual dispersed hepatocytes enriches CLF. These CLF enriching

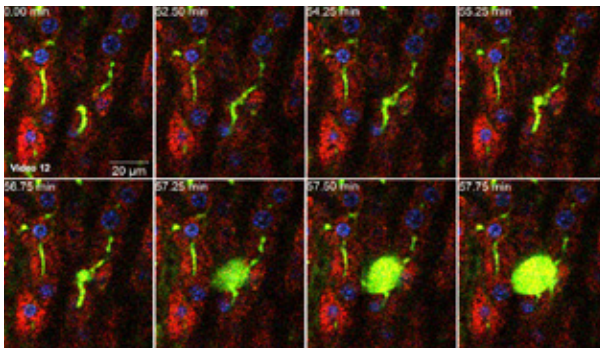


hepatocytes occur at day 1 and 3 after BDL but decrease to very low levels later. Long term imaging demonstrated a 'domino effect', where the neighbours of CLF enriching hepatocytes also begin to accumulate bile salts, finally resulting in an infarct. We next focussed on the individual CLF enriching hepatocytes, the nidus of bile infarcts, to understand the mechanism responsible for bile salt enrichment. Using high magnification and fast sequences of recording the following sequence of events was observed: individual hepatocytes lose their mitochondrial potential; adjacent bile canaliculi form dilatations and protrusions into the cytoplasm of hepatocytes which finally burst and the hepatocyte is flooded with CLF. Interestingly, these hepatocytes intermittently release CLF into the adjacent sinusoids, which exposes neighbouring hepatocytes to increased bile salt concentrations. Further recording shows cell death as evidenced by propidium iodide uptake followed by infiltration of leukocytes. In conclusion, BDL leads to an uptake block of bile salts from sinusoidal blood into hepatocytes, which was observed until day 21 and probably represents a permanent alteration. In contrast, bile infarcts occur only during an acute phase at day 1 and 3 after BDL. Bile infarcts are formed as a sequence of events where initially energy metabolism of individual, dispersed hepatocytes becomes compromised, next the integrity of apical membranes is lost allowing bile regurgitation, followed by a domino effect, where also neighbouring hepatocytes are similarly affected.

Jansen et al., *Hepatology*. 2017 Feb;65(2):722-738; Vartak et al., *Hepatology*. 2016 Mar;63(3):951-64.

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**Figure 1:** Two-photon based intravital imaging of a mouse on day 1 after BDL. The fluorescent bile salt analog CLF is visualized by green fluorescence. The images show a bile canaliculus that forms protrusions, becomes leaky and the adjacent hepatocyte is flooded with CLF. Subsequently, the basolateral membrane is altered and CLF is released to the neighbouring sinusoid.



## Development of the biliary tree

**Frédéric Lemaigre**\*<sup>1</sup>

*<sup>1</sup>de Duve Institute, Université catholique de Louvain, Brussels, Belgium*

**Corresponding author's email:** frederic.lemaigre@uclouvain.be

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The study of liver development using cell culture and animal models has uncovered a number of signalling pathways and transcriptional regulators which drive differentiation of liver progenitor cells (hepatoblasts) towards hepatocytes or cholangiocytes, and subsequent morphogenesis of hepatocyte cords and bile ducts. Recent data in the field of bile duct development raise new questions about the three-dimensional mode of duct morphogenesis, and on quantitative and dynamic aspects of gene regulatory networks.

To characterize how morphogenesis is controlled during cholangiocyte differentiation we analysed 2D and 3D images to propose an updated model of bile duct development. Data from the literature were combined with our own to design a model of bile duct development in which growth of the ducts along their longitudinal axis is associated with differentiation of hepatoblasts to cholangiocytes along their radial axis.

Inactivation or overexpression of microRNAs and signalling mediators at specific stages of hepatocyte and cholangiocyte differentiation revealed the importance of quantitative levels of gene regulators at distinct steps of liver development. Implementation of quantitative computational modelling helped in identifying the mechanisms through which gene regulators control hepatic cell differentiation, and to uncover how specific network motifs contribute to cell fate decision and maturation during development.

We conclude that the dynamics of gene regulatory networks are essential for hepatic cell differentiation and organ morphogenesis and can be unravelled with help of an appropriate combination of computational and experimental approaches.

## Liver injury combined with inhibition of hepatocyte proliferation leads ductular cells to function as facultative stem cells for hepatocytes

Stuart Forbes\*<sup>1</sup>

<sup>1</sup>Edinburgh University, Edinburgh, United Kingdom

**Corresponding author's email:** [stuart.forbes@ed.ac.uk](mailto:stuart.forbes@ed.ac.uk)

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Following injury, the normal liver regenerates through hepatocyte proliferation. In human liver disease, when hepatocyte replication is impaired, ductular reactions proliferate and expand through the parenchyma. The regenerative potential of these ductular cells has been controversial. Most mouse models of liver injury do not inhibit hepatocyte replication effectively and ductular cells do not significantly regenerate hepatocytes. We have used several models to investigate the regenerative potential of ductular cells in mouse models of liver injury when hepatocyte proliferation is inhibited- a clinically relevant scenario.

We have used the AAV8.TBG.Cre system to efficiently heritably label all hepatocytes with RFP. These mice were crossed with  $\beta 1$  integrin<sup>fl/fl</sup> mice resulting in impairment of hepatocyte replication at the same time as RFP labelling. Liver injury was induced with thioacetamide toxin or dietary models (CDE, DDC) for 1-2 weeks followed by recovery up to 4 weeks.  $\beta 1$  integrin floxed mice had reduced hepatocyte proliferation, hepatocyte senescence, marked ductular reactions and the development of non-hepatocyte derived regenerative nodules accounting for 25% of the hepatocytes by 2 weeks recovery. These unlabelled hepatocytes were more proliferative and had smaller nuclei than the original RFP labelled hepatocytes.

We have used a mouse model system to fate map ductular cells into hepatocytes using *Krt19CreER<sup>TL</sup>LSL<sup>tdTomato</sup>* mice combined with either p21 mediated inhibition of hepatocyte replication or  $\beta 1$  integrin deletion from hepatocytes. The large-scale differentiation of the ductular cells into hepatocytes is seen which is dependent upon both liver injury (CDE, DDC) and the inhibition of hepatocyte replication. These systems model clinical liver disease where hepatocyte proliferation is impaired and permit the analysis of mechanisms controlling ductular cell activation and differentiation.

## Repopulating the biliary tree from the peribiliary glands

**Robert J. Porte**<sup>\*1</sup>

*<sup>1</sup>Dept. of HPB Surgery and Liver Transplantation, University Medical Center Groningen, Groningen, The Netherlands*

**Corresponding author's email:** r.j.porte@umcg.nl

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The luminal lining of the biliary tree is constituted of cholangiocytes, or biliary epithelial cells, which vary in phenotype depending on the diameter of the bile ducts. The larger segmental intrahepatic bile ducts as well as the extrahepatic parts of the biliary tree are covered by relative large biliary epithelial cells, compared to the small intrahepatic ductules and canaliculi that are covered by small cholangiocytes. Cholangiocytes (or biliary epithelial cells) not only contribute to bile production by the secretion and reabsorption of various biliary compounds (i.e. bicarbonate secretion), but also provide a physical barrier preventing leakage of bile of biliary components from the biliary lumen into the bile duct wall and outside the ducts. Similar to the mucosal lining of the intestine, the biliary epithelial lumen is constantly renewed. Timely and adequate biliary regeneration requires at least two important preconditions: adequate supply of oxygen and nutrients and a vital source from which biliary epithelial cells can proliferate and regenerate. Regeneration of biliary epithelial cells can take place by proliferation of adult biliary epithelial cells lining the bile duct lumen or by the proliferation of progenitor cells. While renewal of the biliary epithelium of the small bile ducts has been extensively studied, the mechanisms of biliary epithelium regeneration in the larger and extrahepatic bile ducts are not very well known. Most previous research has focused on the smaller, intrahepatic bile ducts and ductules. These studies have indicated a role for the proliferation of remnant adult biliary epithelial cells, but also of bipotent stem cells in the canals of Herring (Oval cells), and bone marrow derived stem cells (1,2). Regarding regeneration of the biliary epithelial lining of the larger bile ducts, proliferation of existing adult biliary epithelial cells at the luminal surface, as well proliferation of cells from the peribiliary glands have been demonstrated to play a role [3- 7]. The peribiliary glands are small circular groups of cholangiocytes nested in the wall of the larger bile ducts, which have been identified as an important niche of biliary progenitor cells (Figure 1). Peribiliary glands are connected to the luminal surface via small canals through which newly formed biliary epithelial cells can migrate and contribute to the restoration of the epithelial lining of the bile duct lumen [6,7]. Progenitor-like cells are mainly found in peribiliary glands that are located in the deeper layers of the bile duct wall, near the fibromuscular layer [5,6]. It has been suggested that inadequate regeneration of cells from the peribiliary glands plays a role in the development of cholangiopathies in which mainly the larger bile ducts of the biliary tree are affected, such as primary sclerosing cholangitis and post-ischemic cholangiopathy. Post-ischemic

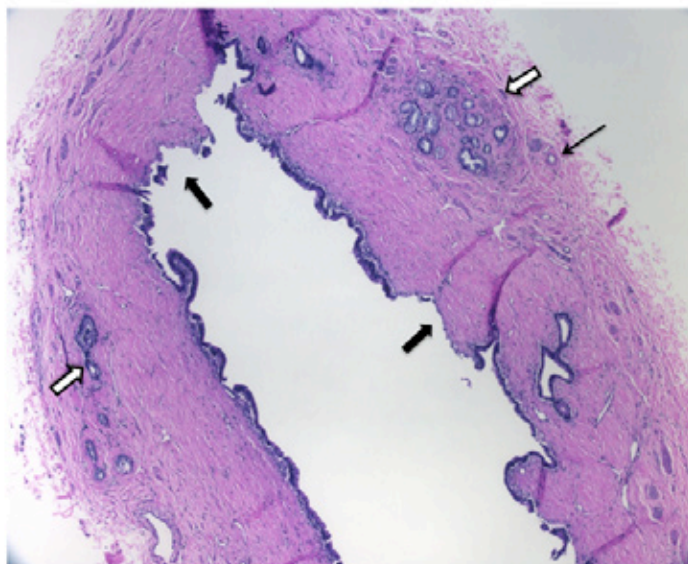
cholangiopathy can be seen after liver transplantation, especially after transplantation of a liver from a DCD (donation after circulatory arrest) donor, where the liver has suffered a period of warm ischemia during circulatory arrest of the donor, followed by cold ischemia during preservation and transportation [8]. This type of cholangiopathy is also known as ischemic-type biliary lesions (ITBL) or strictures or non-anastomotic biliary strictures (NAS) of the larger donor bile ducts. A similar picture of biliary abnormalities after transplantation can be observed after hepatic artery thrombosis. While biliary ischemia is obviously the underlying cause in the latter, ITBL or NAS is typically seen in the absence of arterial perfusion problems of the donor liver. Most cases of NAS become symptomatic within the first six months after transplantation. In a large prospective clinical study of 128 patients undergoing liver transplantation it was recently shown that biopsies taken from the extrahepatic bile duct at the time of transplantation display signs of serious injury of the luminal biliary epithelial lining in 90% of all cases [9]. In contrast with this, only a minority of the donor livers developed NAS after the transplant procedure, suggesting that adequate and timely regeneration of the biliary epithelium occurs in the majority of cases. However, when histological examination of the bile duct at the time of transplantation revealed major injury of the deep peribiliary glands and/or the peribiliary vascular plexus, this was significantly associated with the development of NAS after the transplant procedure. This study contributed to the existing evidence that the peribiliary glands of the larger bile ducts play a critical role in regeneration of the biliary epithelial lining. If the deep peribiliary glands have become seriously damaged during the graft preservation and transplantation, the regenerative capacity of the bile duct epithelium is affected and timely renewal of the lost luminal epithelium does not take place, resulting in the development of sclerosing biliary strictures [9,10].

In conclusion, while bi-potent progenitor cells nested in the intrahepatic canals of Herring have been identified as a source from which the epithelial lining the smaller intrahepatic bile ductules can be reconstituted, the peribiliary glands of the segmental and extrahepatic bile ducts constitute a niche of biliary progenitor cells for these larger bile ducts. Better understanding of the regenerative potential and triggers for proliferation of the peribiliary gland cells may aid in the development new therapies of cholangiopathies of the larger bile ducts.

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**Figure 1.** Light microscopy of a hematoxylin-eosin stained biopsy of the extrahepatic bile duct of a donor liver. Indicated are loss of the luminal epithelial lining (thick black arrows), peribiliary glands (white arrows) and an arteriole of the peribiliary vascular plexus (thin black arrow).

# Bio-engineering the biliary tree

Fotios Sampaziotis\*<sup>1</sup>

<sup>1</sup>University of Cambridge, Cambridge, United Kingdom

**Corresponding author's email:** fotiss@yahoo.com

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## Background and aims

The management of bile duct disorders such as biliary atresia, primary sclerosing cholangitis and biliary strictures is limited to liver transplantation and hepatojejunostomy due to the lack of bile duct tissue suitable for surgical replacement. We have recently developed a method for the isolation and propagation of primary human extrahepatic cholangiocyte organoids (ECOs). Here we explore the potential of these organoids for populating biodegradable scaffolds and generating bio-engineered biliary tissue which is then used for biliary reconstruction in vivo.

## Methods

Primary adult human cholangiocytes were isolated by mechanical dissociation from deceased organ donors with appropriate ethical approval and informed consent (n=8). Propagation of the cells was achieved using our established protocol for maintenance of ECOs. Transcriptomic characterization (microarrays) was performed using the Illumina HumanHT-12 v4 Expression BeadChip Array. To generate biliary tissue ECOs were dissociated to single cells, seeded on Polyglycolic Acid (PGA) or densified collagen tubes and expanded for up to 4 weeks until the scaffolds were confluent. Biliary reconstruction was achieved by partially removing the gallbladder wall and repairing the defect with an ECO populated PGA-scaffold patch (ECO-patch; n=8), or excising a length of the native common bile duct (CBD) and replacing it with an ECO populated collagen tube (ECO-tube) through end-to-end anastomosis (n=11). All experiments were performed in immune compromised mice. Fibroblast-populated (n=5 PGA scaffolds; n=4 collagen tubes) or acellular scaffolds (n=2, PGA) were used as negative controls. The patency of the reconstructed biliary tree was confirmed using magnetic resonance cholangiopancreatography (MRCP) or cholangiogram.

## Results

ECOs express key biliary markers such as CK7, CK19, GGT, CFTR and closely correlate with primary cholangiocytes in terms of transcriptomic profile ( $r:0.92$ ), and functional properties (ALP, GGT, bile acid transfer, response to secretin/ somatostatin stimuli). Importantly, ECOs exhibit a linear growth curve for multiple passages and maintain their genetic stability on karyotyping and CGH analyses. When transplanted under the kidney capsule of immune compromised mice, ECOs self-organized into tubular structures

maintaining expression of biliary markers (CK7, CK19). When seeded on biodegradable scaffolds ECOs formed biliary tissue-resembling structures, maintaining their functional properties (GGT, ALP activity) and marker expression. Furthermore, ECO-tubes maintained a patent lumen even after long-term culture. All animals undergoing biliary reconstruction with ECO-populated scaffolds exhibited prolonged survival (PGA vs. acellular controls,  $P=0.0027$ ; Collagen tubes vs. fibroblasts,  $P=0.0082$ ; log-rank test). The transplanted cells were integrated in the biliary epithelium, continued expressing biliary markers (CK7, CK19, HNF1B) and exhibited ALP activity. The patency of the reconstructed gallbladder or the replaced bile duct was confirmed using MRCP. All reconstructions with fibroblast-populated scaffolds failed, the biliary epithelium was replaced by fibrotic tissue and the lumen of the gallbladder or neo-bile duct was occluded.

### **Discussion**

We demonstrate that ECOs demonstrate a unique potential for regenerating the biliary epithelium. Furthermore, ECO-populated biodegradable scaffolds maintain their biliary identity *in vitro* and *in vivo* and can reconstruct/replace parts of the biliary tree following transplantation. To our knowledge, this is the first demonstration for the application of regenerative medicine in the management of cholangiopathies and first report of an organ reconstruction using human primary cells expanded *in vitro*.



## Animal models of biliary injury and altered bile acid metabolism

Diego F. Calvisi<sup>1</sup>, Luca Fabris<sup>\* 2, 3</sup>

<sup>1</sup>Institute of Pathology, University of Greifswald, Greifswald, Germany, <sup>2</sup>Department of Molecular Medicine, University of Padua, Padova, Italy, <sup>3</sup>Digestive Disease Section, University of Yale, New Haven (CT), United States

Corresponding author's email: [luca.fabris@unipd.it](mailto:luca.fabris@unipd.it)

In the last 25 years, a number of animal models (mainly developed in rodents) have been generated with the goal to mimic cholestatic liver injuries and, thus, to provide *in vivo* tools to investigate the mechanisms of biliary repair and, eventually, to test the efficacy of innovative treatments. Although fundamental limitations apply to these models, such as the distinct immune system and the different metabolism regulating liver homeostasis in rodents when compared to humans, the ability of these animal models to reproduce critical facets of cholestatic liver diseases hitherto represents a valuable asset.

Surgical bile duct ligation (BDL) in mice and rats is the most widely used model of biliary fibrosis determined by acute obstructive cholestasis. BDL animals promptly develop jaundice accompanied by typical biliary lesions, including intense ductular reaction and portal inflammation with rapid establishment of portal fibrosis. Taking a different approach of chemically-induced cholangitis, in the 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-fed mice, biliary damage was caused by an increased biliary porphyrin secretion resulting in segmental bile duct obstruction. The main features of this animal model are a brisk ductular reaction, associated with pericholangitis, and periductal fibrosis finally evolving by 8 weeks to portal-portal bridging, driven by a dense periductal accumulation of activated myofibroblasts (1).  $\alpha$ -naphthylisothiocyanate (ANIT)-fed mice is another model of biliary injury induced by xenobiotics. ANIT is a chemical compound undergoing hepatocyte metabolism, through glutathione conjugation and bile transport by the canalicular pump Mrp2 (2). Since glutathione-conjugated ANIT is highly unstable in the bile, it is continuously recycled by hepatocytes, leading to progressive increase in bile levels that become toxic for cholangiocytes. The phenotype is characterized by a pronounced bile duct hyperplasia, periportal inflammation and, finally, biliary fibrosis (3). Alternatively, several genetically engineered rodent models have been established to study biliary damage. Among them, the *Mdr2* (ATP binding cassette subfamily B member 4 or *Abcb4*) gene knockout (KO) mouse has gained considerable interest as surrogate model of primary sclerosing cholangitis (PSC). *Mdr2* defect leads to a defective excretion of phosphatidylcholine into the bile, which increases the biliary cholesterol saturation index. These chemical alterations in bile composition trigger non-purulent cholangitis with pronounced ductular proliferation, starting early after birth and progressing through

biliary cirrhosis by 3 to 6 months (4). Interestingly, in contrast to *Mdr2*-KO mice, bile salt export pump (*BSEP*)-KO mice do not develop severe cholestasis, due to the replacement of the bile acid pool with more hydrophilic bile acids, including muricholic acid and atypical bile acid species, which are not produced in humans. Consequently, these animals do not show any histopathologic sign of liver injury unless treated with cholic acid (5).

Altogether, the aforementioned animal models have raised critical concerns in terms of translatability into clinic, particularly with respect to the severity and the celerity of the induced fibrogenesis, which is scarcely consistent with what observed in humans. In BDL, liver cirrhosis develops after only 30 days, at odds with the slow progression of most chronic liver diseases, particularly cholangiopathies, usually spanning over decades. Furthermore, in *Mdr2*-KO mice the causative deficiency affects the hepatocyte rather than the cholangiocytes; thus, it does not address a specific dysfunction of the biliary epithelium.

Therefore, mice harboring genetic inactivation of proteins normally expressed by cholangiocytes offer a more fascinating prospect to unravel the complex scenario of biliary repair as it occurs in humans. In particular, targeting specific proteins involved in biliary ontogenesis enables us: a) to understand the morphogenetic pathways, which are recapitulated in biliary repair; b) to investigate with a 'tidy' approach selective repair mechanisms, without the interference of multiple pathogenetic factors typical of acquired conditions, as the immune-mediated (primary biliary cholangitis, PSC) or the infectious biliary diseases. In this sense, paradigmatic are mice with defects in genes coding for membrane channel transport proteins, such as the cystic fibrosis transmembrane conductance regulator (CFTR), or for ciliary proteins, such as polycystins (polycystin-1 and polycystin-2) and fibrocystin. While CFTR dysfunctions affects not only bile production, but also innate immune pathways (6, 7), defects in polycystins and fibrocystin result in the persistence of biliary cells with an immature, fetal-like phenotype (ductal plate malformations), that, in the post-natal life, leads to dysgenetic bile ducts. Their ability to secrete a range of pro-inflammatory mediators leads to unrestrained repair responses featuring bile duct proliferation, in conjunction with peribiliary inflammation and fibrosis (8, 9). The 'core' pathways identified in cholangiocytes presumably regulate repair mechanisms in various contexts of liver injury. Thus, these experimental models may provide a wealth of information going beyond the scenario of genetic cholangiopathies.

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# Mechanisms of regulation of cholangiocyte response to injury

Gianfranco D. Alpini\*<sup>1</sup>

<sup>1</sup>Medicine, VA and Texas A and M Health Science Center, Temple, United States

Corresponding author's email: galpini@tamu.edu

Cholangiocytes are the targets of cholangiopathies, including primary sclerosing cholangitis (PSC) that are identified by dysregulation of cholangiocyte proliferation/apoptosis alongside increased portal inflammation, biliary damage and liver fibrosis. Cholangiocytes are normally mitotically dormant, but markedly proliferate following bile duct ligation (BDL, model of cholestasis) and in the *Mdr2*<sup>-/-</sup> mouse model of PSC. Biliary proliferation is coordinately regulated by a number of neuroendocrine factors including secretin (Sct). Sct is a gastrointestinal peptide that acts through its specific receptor, SR that in the liver is expressed only in cholangiocytes. Since SR is cholangiocyte-specific, differential expression and/or activation of Sct-dependent signaling pathway is a pathophysiological tool for evaluating cholangiocyte-induced damage. We have shown that (i) cholangiocyte proliferation (following BDL and in *Mdr2*<sup>-/-</sup> mice) is associated with enhanced SR expression and (ii) loss of the secretin/SR axis (through knockout mouse models or an SR antagonist) reduces biliary hyperplasia and liver fibrosis associated with cholestatic injury. Specifically, following cholestasis there enhanced expression of the Sct/SR axis in cholangiocytes, which reduces the expression of the microRNAs miR-125b and let-7a, resulting in increased levels of vascular endothelial growth factor (VEGF) and nerve growth factor (NGF) concomitant with enhanced liver fibrosis. We have shown that VEGF and NGF contribute to biliary proliferation and liver fibrosis, providing mechanistic detail as to how secretin regulates liver damage. Aside from microRNA regulation, we have shown that following BDL and in *Mdr2*<sup>-/-</sup> mice the Sct/SR axis increases the biliary expression of various pro-fibrotic markers, including transforming growth factor (TGF)-b1. Furthermore, loss of this signaling axis through knockout mouse models or SR antagonist treatment leads to reduced TGF-b1 expression and liver fibrosis. Aside from cholangiopathies, the role of cholangiocytes in other liver diseases is becoming an area of interest. Nonalcoholic fatty liver disease (NAFLD) is derived from simple steatosis and develops to nonalcoholic steatohepatitis (NASH). Studies have identified a cholestatic variant of NAFLD with ductular reaction, and these patients are more susceptible to developing bridging fibrosis later on. We hypothesize a role for cholangiocytes in the progression of NAFLD/NASH. To evaluate our hypothesis, we fed wild-type (WT) or *SR*<sup>-/-</sup> mice a high fat, trans fat diet (HFD) supplemented with 0.2% cholesterol and a high fructose corn syrup equivalent to closely mimic a typical Western diet. We found that *SR*<sup>-/-</sup> HFD mice had significantly reduced hepatic steatosis when compared to WT HFD mice.

As well, we found that cholangiocytes from SR<sup>-/-</sup> HFD mice had increased expression of lipid metabolism genes as compared to WT HFD mice. These findings introduce a new role for cholangiocytes in the development of hepatic steatosis through modulation of lipid metabolism. As well, WT HFD mice had enhanced biliary proliferation, and as expected SR<sup>-/-</sup> HFD mice had reduced biliary proliferation when compared to WT HFD mice. Alongside increased biliary proliferation, WT HFD mice had enhanced senescence and lipoapoptosis. Oppositely, these factors were reduced in SR<sup>-/-</sup> HFD mice. This further defines the concept that cholangiocytes are a heterogeneous population wherein one subset may present with enhanced senescence and/or lipoapoptosis, which may influence neighboring cholangiocytes to enter a proliferative state. In Mdr2<sup>-/-</sup> mice we have found that large cholangiocytes have increased senescence while small cholangiocytes have reduced senescence and become more proliferative. Aside from cholangiocyte proliferation, SR<sup>-/-</sup> HFD mice had decreased hepatic fibrosis and TGF- $\beta$ 1 levels. Herein, we present novel work identifying the key role that cholangiocytes play during HFD induced damage. Further understanding the autocrine/paracrine signaling pathways by which these effects are being mediated is being studied. As well, identifying the role that SR may play during insulin resistance and metabolic syndrome is an important area of interest. Early work from us has shown that cholangiocytes express the insulin receptor, implicating a key role for cholangiocytes during metabolic syndrome and insulin resistance. Furthermore, others have shown that cholangiocytes express glucose transporters and respond to glucose found in bile, cementing the concept that cholangiocytes are active participants in the development and progression of NAFLD/NASH and metabolic syndrome. Highlighting the major impact that this small population of cells has during NAFLD/NASH development provides new insights and potential therapeutics for this endemic disease.

## Non coding-RNAs and extracellular vesicles

Jesus M. Banales\*<sup>1</sup>

<sup>1</sup>Liver Diseases Group, Biodonostia Health Research Institute, San Sebastian, Spain

**Corresponding author's email:** [jesus\\_banales@yahoo.es](mailto:jesus_banales@yahoo.es)

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Cholangiopathies are a group of diseases targeting the biliary epithelial cells (i.e. cholangiocytes). These disorders encompass a wide spectrum of etiologies comprising genetic, infectious, immune-mediated, drug-induced, vascular, neoplastic or idiopathic. Although their pathogenesis remain still obscure, chronic inflammation and cholestasis seem to be common events that exacerbate the wound-healing response leading to the development of liver fibrosis and cirrhosis. Most cholangiopathies lack valid diagnostic and/or prognostic biomarkers, as well as adequate targets for therapy, demanding the need of further research.

In the last years, the discovery of microRNAs (miRNAs) has represented a revolution and a paradigm shift, postulating them as promising biomarkers and targets/tools for therapy. These small (18-23 nucleotides) endogenous non-coding RNAs play significant roles in most physiological and pathological cellular events including proliferation, differentiation, migration, senescence and survival, by post-transcriptional regulation of gene expression. Cholangiopathies display aberrant miRNA signatures in cholangiocytes, immune cells, liver tissue and biological fluids among others, evidencing the potential value of miRNAs in diagnosis, prognosis and therapy. MiRNAs can be released into the extracellular medium associated with lipoproteins or encapsulated in extracellular vesicles (EVs), thus participating in intercellular communications.

EVs are small lipid-enclosed spheres secreted by many cell types and found in multiple biological fluids including bile, blood and urine. There are different types of EVs according to their origin, size, molecular composition and biological function. Regarding their origin, EVs are classified into exosomes (40-200 nm), plasma membrane-derived vesicles (40- >1000 nm) and apoptotic bodies (100 – 5000 nm). Besides miRNAs, they can also contain other nucleic acids, lipids and proteins. To date, the most studied EVs are exosomes, which are released extracellularly upon exocytic fusion of endosome-derived multivesicular bodies (MVBs) with the plasma membrane of the cells. Then, the exosomal content can be further delivered into recipient cells where it can regulate gene expression and cellular functions. Changes in the transcriptomic and proteomic EV content has been reported in different cholangiopathies, pointing out their potential value as non-invasive biomarkers.

In sum, cholangiopathies represent a global health problem that warrants considerable attention and thorough investigation. This presentation aims to provide current knowledge on the role of miRNAs and EVs in the pathogenesis of biliary diseases, and their potential

therapeutic value. Moreover, their present impact as new non-invasive diagnostic and prognostic tools, and their value in personalized patient care, will be discussed. In this regard, recent studies have described a significant number of promising candidate miRNAs and EV-related biomarkers that need to be validated in upcoming international collaborative studies. Finally, future directions on basic and clinical investigations will be highlighted.

# Genetics and mechanisms of biliary cystogenesis: Impact in the clinical practice

**Joost Ph Drenth<sup>\*1</sup>**

*<sup>1</sup>Gastroenterology and Hepatology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands*

**Corresponding author's email:** [joostphdrenth@cs.com](mailto:joostphdrenth@cs.com)

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Polycystic liver disease (PLD) is the primary phenotype of autosomal dominant polycystic liver disease (ADPLD) and is present in the majority of autosomal dominant polycystic kidney disease patients (ADPKD). Almost all ADPKD patients harbour mutations in genes encoding Polycystic Kidney Disease 1 (PKD1) or Polycystic Kidney Disease 2 (PKD2). Mutations in 4 genes explain collectively ~ 25% of the genetic background of ADPLD. One gene, LRP5 encodes a key component of the LRP5/LRP6/Frizzled co-receptor group and is involved in canonical Wnt pathway, while SEC63p is part of the protein translocation apparatus in the endoplasmic reticulum (ER) membrane. Glucosidase II complex is encoded by 2 genes (PRKCSH and GANAB and aid in glycoprotein processing and quality control in the ER. Mutations in either gene are seen in PLD patients.

The molecular mechanism that underlies cystogenesis is the accumulation of multiple somatic mutations in a population of cells. This concept assumes that presence of a germline mutation ('first hit') in an inherited disorder requires a 'second hit' at the somatic level for cyst development to occur. The second hit is the rate-limiting step and results in somatic inactivation of the normal allele. Studies have identified secondary, somatic hits in PLD cyst tissues in ADPLD and ADPKD. Inactivation of the wild type allele occurs through somatic mutations or loss of heterozygosity (LOH). LOH is caused by either terminal copy number neutral (CNN) LOH or interstitial deletions, particularly in case of patients with a PRKCSH germline mutation. This concept is also valid for sporadic liver cysts, but there both alleles are lost through somatic mutations. While ADPLD and ADPKD are regarded as two distinct genetic disorders, mutations in GANAB may be associated with both ADPKD and ADPLD. Recent evidence suggests that defects in SEC63, GANAB and PRKCSH result in decreased levels of functional polycystin-1 and -2 complex. Functional polycystin-1 determines cyst formation and cyst growth can be accelerated or mitigated depending on polycystin-1 dosage.

# Pathogenesis and 3D Structure of Biliary Fibrosis

**Massimo Pinzani**<sup>1</sup>

<sup>1</sup>*Sheila Sherlock Chair of Hepatology, Director, UCL Institute for Liver and Digestive Health, London, United Kingdom*

**Corresponding author's email:** [m.pinzani@ucl.ac.uk](mailto:m.pinzani@ucl.ac.uk)

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Chronic liver diseases affecting bile ducts, namely primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), are characterized by the development of the so-called biliary fibrosis. In general terms, biliary fibrosis originates from portal tracts and tends to expand with a portal-to-portal pattern. In more advanced stages, fibrosis extends to the lobular area and eventually leads to the development of biliary-type cirrhosis. It is important to stress that, in both PBC and PSC, the fibrogenic process is consequent to the involvement of small bile ducts within portal areas. In PBC, bile ducts are infiltrated by immune/inflammatory cells causing structural damage, ductopenia and peri-biliary stromal expansion. In PSC the early features are represented by a peri-biliary inflammatory reaction which progressively causes concentric “onion-like” fibrosis with a progressive obliteration of the bile ducts. The progressive structural damage of the intrahepatic biliary tree leads to cholestasis, which has been traditionally considered an important pro-fibrogenic factor. Indeed, bile salts are classically regarded as “cytotoxic” and their accumulation in liver tissue is thought to play a key role in determining liver cell necrosis and, then, in sustaining the development of liver fibrosis. However, this assumption is based on studies performed on cultured hepatocytes by employing concentrations of bile salts in general largely exceeding the pathophysiological range. In addition, some studies have suggested that the accumulation of bile salts in liver tissue is responsible for a certain degree of hepatocellular apoptosis rather than necrosis. Overall, it appears that “pure” intrahepatic cholestasis in the absence of hepatocellular damage, lobular inflammation and bile duct damage and/or proliferation, is not associated with marked and/or progressive liver tissue fibrosis. Although it could be argued that most causes of intrahepatic cholestasis are generally short-lasting (i.e. drug-induced), or not continuous (i.e. benign recurrent cholestasis) it is quite evident that a significant fibrogenic reaction occurs only when the pathological process involves the structure of the bile duct and the cellular phenotype of cholangiocytes. In particular, cholangiocytes can become the source of pro-inflammatory and pro-fibrogenic factors assuming a phenotype defined “reactive”. Indeed, evidence obtained employing *in vitro* cultures of cholangiocytes as well derived from *in situ*/ICH of human liver samples from PSC liver, suggests that reactive cholangiocytes are able to express pro-fibrogenic factors such as PDGF-BB, TGF- $\beta$  1, MCP-1, Endothelin-1 and many others. Interestingly, the morphology and the 3D structure of liver fibrosis typical of PSC seem to confirm a pro-fibrogenic focus originating from the cholangiocyte layer



leading to the concentric recruitment and activation of pro-fibrogenic cells from the portal stroma and from the neighboring lobular area. Finally, emerging evidence suggests that, in addition to marked difference in the 3D structure of different types of biliary fibrosis, there are also large variances in the biochemical composition of the fibrotic ECM as indicated by recent proteomic analysis employing laser microdissection and decellularised ECM scaffold technology.

## Nuclear hormone receptors as drug targets

**Bart Staels<sup>\*1</sup> and Univ. Lille - European Genomic Institute for Diabetes (E.G.I.D); INSERM UMR 1011; CHU Lille; Institut Pasteur de Lille, France**

<sup>1</sup>*GENFIT SA, Lille, France*

**Corresponding author's email:** bart.staels@pasteur-lille.fr

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Bile acids (BA) exert important functions in the entero-hepatic system. BA are synthesized and conjugated in the liver, secreted in the duodenum after meal ingestion, modified by the intestinal gut flora, reabsorbed in the intestine and transported back to the liver by the portal vein. Although most BA are recaptured by hepatocytes, a fraction escapes and reaches peripheral organs.

BA pool size and composition are modulated by metabolic perturbations, being altered in obesity, insulin-resistance, type 2 diabetes and non-alcoholic steatohepatitis (NASH) in preclinical models and humans. BA sequestrants, which interrupt the entero-hepatic BA circulation, improve lipid/glucose homeostasis. Modification of the intestinal microbiota also alters the BA pool in mice. Systemic BA concentrations increase after Roux-en-Y-gastric-bypass (RYGB) surgery in humans and animal models.

BA modulate metabolism in part through activation of the nuclear receptor Farnesoid X Receptor (FXR) and the membrane receptor TGR5. BA and FXR regulate metabolism via entero-hepatic signalling. FXR protects the liver from BA overflow by regulating BA synthesis, secretion and transport. In the intestine, BA bind to FXR in ileal enterocytes to induce FGF15/19 expression which in turn inhibits hepatic BA synthesis. In the liver, FXR also regulates glucose and lipid metabolism. Moreover, FXR signalling contributes to the changes in gut microbial communities and the metabolic benefits of vertical sleeve gastrectomy. Furthermore, microbiota-modified BA not only act in the gut, but also on the entero-hepatic system to control hepatic BA metabolism and obesity. Intestinal FXR signalling also modulates NASH. We recently reported FXR expression in the entero-endocrine tissue, specifically in entero-endocrine L cells, where FXR inhibits the response to glucose on the production and secretion of the incretin GLP-1. These studies established the importance of BA signalling through FXR in the intestine and the potential of FXR as a target in the treatment of cholestatic and metabolic diseases.

## Gut and liver interactions

**Antonio Moschetta**<sup>\*1</sup>

<sup>1</sup>*Department of Interdisciplinary of Medicine, Clinica Medica "Frugoni", Universita Degli Studi di Bari - Policlinic, Bari, Italy*

**Corresponding author's email:** antonio.moschetta@gmail.com

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While it has long been recognized that bile acids (BAs) are required to facilitate lipid digestion and cholesterol absorption in the intestine, the discovery that they serve as ligands for the nuclear Farnesoid X receptor (FXR), opened a new chapter in the characterization of BAs as signalling molecules. FXR is a transcription factor and the master regulator of BAs homeostasis. In the gut-liver axis, FXR regulates BA synthesis, influx, efflux, and detoxification. The landmark discovery of the role of a FXR target gene, the fibroblast growth factor 15/19 (murine and human, respectively – FGF15/19), in the feedback regulation of hepatic BA synthesis shed light on the physiological relevance of the crosstalk of the gut-liver axis in the context of BA homeostasis. FGF15/19 is an atypical fibroblast growth factor that functions as a hormone. Via the enterohepatic circulation, it travels from the small intestine to the liver, where it acts on a cell surface receptor complex to repress BA synthesis and gluconeogenesis and activate glycogen and protein synthesis. Deregulation of BA homeostasis has been linked to cholestasis and hepatocellular carcinoma (HCC), and spontaneous hepatocarcinogenesis has been observed in FXR-null mice. We have demonstrated that intestinal selective FXR reactivation is sufficient to restore the enterohepatic BA homeostasis via fibroblast growth factor 15 (FGF15)/cholesterol-7alpha-hydroxylase (Cyp7a1) axis and eventually provide protection against progression of liver damage, even in the absence of hepatic FXR. Intestinal-selective FXR modulators could stand as potential therapeutic intervention in patients with impaired BA homeostasis-associated hepatic diseases, even in patients carrying a somatic FXR mutation.

## TGR5 in physiology and disease

Verena Keitel<sup>1</sup>

<sup>1</sup>*Clinic for Gastroenterology and Hepatology, Heinrich Heine University Duesseldorf, University Hospital Duesseldorf, Duesseldorf, Germany*

**Corresponding author's email:** verena.keitel@med.uni-duesseldorf.de

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### TGR5 in physiology and disease

Kathleen Deutschmann, Maria Reich, Lina Spomer, Caroline Klindt, Dieter Häussinger and **Verena Keitel**

Clinic for Gastroenterology, Hepatology and Infectious Diseases, University Hospital Duesseldorf, Moorenstrasse 5, 40225 Duesseldorf, Germany

TGR5 (Gpbar1) is a G-protein coupled receptor responsive to bile acids, which is expressed in various tissues, including liver (1). In liver, TGR5 has been detected in different non-parenchymal cells, such as cholangiocytes, Kupffer cells, sinusoidal endothelial cells (LSCs) and activated stellate cells (HSC) (1).

In cholangiocytes, TGR5 is localized in the primary cilia, in the apical membrane as well as in intracellular vesicular structures (2,3). Activation of the receptor triggers chloride and thus fluid secretion, thereby enhancing biliary bile flow. In non-confluent cells, TGR5 promotes bile acid induced cell proliferation independent of adenylate cyclase activation through elevation of reactive oxygen species, stimulation of Src kinase and transactivation of the epidermal growth factor receptor, resulting in phosphorylation of ERK1/2 (4). Furthermore, absence of TGR5 from cholangiocytes renders the cells more susceptible towards bile acid induced cell injury and death. The protective role of TGR5 in cholangiocytes was further analysed using TGR5 knockout mice, which were subjected to different cholestatic models. Feeding of cholic acid (CA), lithocholic acid (LCA) or common bile duct ligation triggered cholangiocyte proliferation only in TGR5 wildtype mice but not in TGR5 knockout littermates.

In human liver, TGR5 is also highly expressed in cholangiocytes and the receptor is overexpressed in cholangiocarcinoma (CCA) tissue (4). In cholangiocarcinoma cell lines (EGI-1 and TFK-1) TGR5 stimulation induced cell proliferation, apoptosis resistance and promoted cell migration and invasiveness. Thus, the receptor may contribute to CCA progression. In contrast TGR5 protein levels as measured by immunofluorescence intensity staining are reduced in cholangiocytes of livers from patients with primary sclerosing cholangitis (PSC). This finding was in line with data from Mdr2 (Abcb4) knockout mice, which serve as animal model of PSC, suggesting that the receptor may also play a role in human biliary diseases.



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## Bile acids, FGF15/19 and liver regeneration: from mechanisms to clinical applications

Matias A. Avila\*<sup>1</sup>

<sup>1</sup>Hepatology, CIMA. CIBERehd. Idisna. University of Navarra, Pamplona, Spain

Corresponding author's email: [maavila@unav.es](mailto:maavila@unav.es)

The liver fulfils a wide range of metabolic and secretory functions that are essential for systemic homeostasis. One key hepatic activity is the metabolism of endogenous and exogenous compounds that need to be biotransformed in order to be excreted out of the organism. This function entails a prominent exposure of the hepatocyte to noxious stimuli generated during the detoxification process, which may eventually lead to cellular death. To avoid loss of hepatic function induced by toxicants, as well as by injury caused by hepatitis virus infection, organisms have evolved a remarkable liver regenerative response to guarantee survival. Understanding the essential components of this response may allow the development of hepatoprotective and pro-regenerative therapeutic strategies in acute and chronic liver injury. Liver regeneration has been extensively studied over the past century using experimental models of partial liver resection and chemical liver injury, with clinical findings confirming its conservation in humans. Recovery of liver mass starts rapidly after parenchymal loss, and precisely cessates when the size of functional parenchyma meets the needs of the organism, an intrinsic property known as the “hepatostat”. The exact nature of the signals and mechanisms involved in this process is not completely known. However, some molecules and processes have been identified to play a central role in the tight control of activation and termination of liver regeneration. Most of these molecules fall into three categories according to their chemical nature and biological properties, comprising cytokines, growth factors and metabolites. These molecules can be originated within the regenerating parenchyma, but may also derive from other tissues and reach the liver through the circulation, including the portal circulation. Being the liver a central metabolic organ it is conceivable that fluctuations in the levels of certain metabolites could influence, *i.e.* trigger and stop, liver growth upon damage or resection, and also maintain a liver size that meets the body needs. Among these metabolites bile acids (BAs) have attracted much attention in the past decade. This emerging role for BAs may be related to their activity as signalling molecules, capable of binding and activating nuclear receptors such as farnesoid X receptor (FXR), or the cell surface G-protein coupled receptor TGR5. One of the target genes of FXR is fibroblast growth factor 19 (FGF19, Fgf15 in rodents). FGF19 expression is triggered in ileal enterocytes during the enterohepatic circulation of BAs, and reaches the liver through the portal blood supply. FGF19 exerts potent regulatory effects on glucose, lipid and protein metabolism in hepatocytes, together with a potent inhibition of BA synthesis. FGF19 has also been identified as an important



mediator of the liver regenerative response, being part of the “hepatostat”. In this presentation we will review the current evidences linking BAs to the regulation of the liver regenerative response and the role of FGF19 in this process. Moreover, we will also discuss the potential of engineered FGF19-based molecules as hepatoprotectants and stimulators of liver regeneration in different clinical contexts.

## The molecular mechanism of cholestatic itch

**Ronald Oude Elferink<sup>1</sup>, Ruth Bolier<sup>1</sup>, Jacqueline Langedijk<sup>1</sup>, Andreas Kremer<sup>1</sup>, Dagmar Tolenaars<sup>1</sup>, Catherine W.<sup>2</sup>, Ulrich Beuers<sup>1</sup>**

*<sup>1</sup>Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands, <sup>2</sup>Maternal and Fetal Disease Group, Institute of Reproductive and Developmental Biology, Imperial College London, London, United Kingdom*

**Corresponding author's email:** r.p.oude-elferink@amc.uva.nl

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Itch (pruritus) is a prominent and distressing symptom of many cholestatic liver diseases such as primary biliary cholangitis, primary sclerosing cholangitis but also inherited cholestatic disease like progressive familial intrahepatic cholestasis and Alagille's syndrome but also intrahepatic cholestasis of pregnancy. The mechanism of itch induction is not understood. In the past, it has been hypothesized that increased serum opioid levels may play a role in cholestatic itch perception, but no proof for this was found in patients, apart from the observation that in some patients, opioid antagonists induce signs of opioid withdrawal. Whatever the mechanism may be, cholestatic itch is difficult to treat and no single treatment exists that is effective in all patients. Rifampicin has beneficial effects in a subgroup of patients, but the mechanism by which this acts is unknown. An important observation with regard to the mechanism is that interruption of the enterohepatic circulation (e.g. by surgical diversion, nasobiliary drainage or MARS treatment) leads to rapid resolution of itch. This treatment is, however, invasive and represents a burden to the patient.

On the basis of the effectiveness of biliary diversion, we hypothesized that patients with cholestasis accumulate compounds in the systemic circulation that either directly or indirectly induce itch perception. To test this hypothesis, we screened sera from cholestatic itchy and non-itchy patients for its capacity to stimulate neuroblastoma cells. This screening revealed increased calcium signalling by cholestatic itchy vs. non-itchy sera. Analysis of the active compound pointed towards lysophosphatidic acid (LPA). LPA in serum is formed from lysophosphatidylcholine by the enzyme autotaxin (which is a lysophospholipase D). We could show that serum autotaxin activity in itchy cholestatic patients was significantly increased compared to non-itchy patients suggesting that increased serum autotaxin levels play a role in the generation of cholestatic itch (1).

We have subsequently set up an assay for scratching behaviour in mice. Intradermal injection of LPA in mice caused a dose-dependent scratch behaviour.

More recently, it was reported that transgenic mice overexpressing the bile salt and neurosteroid receptor TGR5 have increased spontaneous itch. Moreover, mice with a disruption of the Tgr5 gene display reduced scratch behaviour upon intradermal injection of the unconjugated bile salt deoxycholate (2). It must be borne in mind that bile salts





are not the only ligands for TGR5, which is also activated by several neurosteroids. Interestingly, it was recently shown that in ICP several sulphated progesterone metabolites accumulate in serum, which not only may play a role in the generation of cholestasis but also are capable of activating TGR5 (3). Activation of TGR5 would be linked to opening of the TRPA1 channel, which has been shown to play a role in itch signal transduction (4). It was very recently also reported that in sensory neurons activation of LPA receptor 5 (LPAR5) is also linked to opening of TRPA1 (5). Hence, TRPA1 may play a central role in itch signal transduction via several routes.

In conclusion, several routes of induced itch signal transduction are presently being explored. In vivo experiments with proper antagonists for the proposed targets will learn which of these pathways are relevant for cholestatic itch.

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## Bile acid-induced nephropathy

**Peter Fickert**<sup>\*1</sup>

*<sup>1</sup>Medical University Graz, Department of Internal Medicine, Division of Gastroenterology and Hepatology, Graz, Austria*

**Corresponding author's email:** peter.fickert@medunigraz.at

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Acute kidney injury (AKI) is common in patients with liver disease and associated with significant morbidity and mortality. Several prospective studies have shown that classification of AKI in patients with liver cirrhosis based on the kidney disease improving global outcome criteria (KIDIGO criteria) predicts patient mortality in a stage-dependent fashion. Most importantly, the CANONIC study recently showed that impaired renal function plays a central role in defining acute-on-chronic liver failure (ACLF). Taken together these results demonstrated the outstanding prognostic importance of renal function in patients with advanced liver diseases.

In contrast to the previous concept of pure functional hepatorenal syndrome (HRS) more recent pathophysiologic concepts of kidney injury in cirrhosis include structural kidney damage caused by ischemia and inflammation (1). Recent studies do therefore not primarily support the traditional concept that HRS represents exclusively a functional disorder, which is not related to any structural disorders or tissue injury. Again, results of the pivotal CANONIC study showed that AKI was associated at first with systemic inflammation and not that much with cardiocirculatory dysfunction. Consequently, our traditional concepts on extrahepatic organ dysfunction and failure in patients with advanced liver diseases especially in regard to HRS are labile. Therefore, current research on the pathophysiology of AKI in patients with advanced liver disease concentrates on the potential importance of DAMPs and PAMPs. However, we are just at the beginning to understand the complex interplay of gut integrity, intact immune system, liver function, and related kidney alterations. The recent change of diagnostic criteria and management algorithm of renamed AKI-HRS in patients with liver diseases may much better pay attribute to the clinical reality in that this is usually a multifaceted problem (2).

Besides bacterial infections, overdiuresis, use of nephrotoxic drugs, and gastrointestinal blood loss AKI in severe liver disease may also be triggered by tubular toxicity of cholephiles (1) (summarized in Fig.1.). Cholemic nephropathy (CN) represents a probably widely underestimated but important cause of renal dysfunction in patients with prototypic cholestatic liver diseases and advanced liver diseases with cholestasis due to secretory failure of the liver (3). CN and its synonyms (e.g. icteric nephrosis/nephropathy, jaundice-related nephropathy, bile cast nephropathy) have almost disappeared from pathology textbooks and modern medical literature. However, neglecting the potential contribution of these phenotypical renal changes to the clinical spectrum of AKI especially in the case

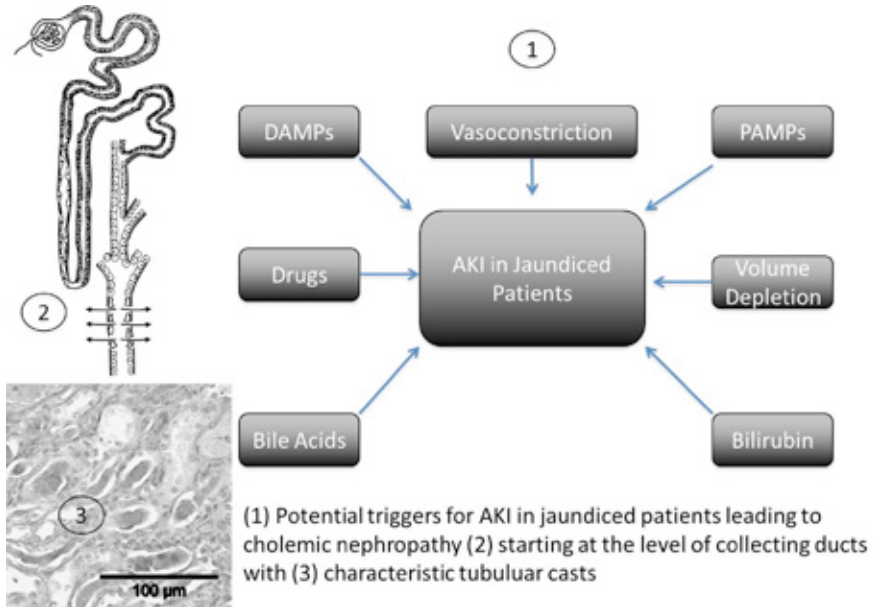
of cholestatic liver diseases or deeply jaundiced patients with ASH or liver cirrhosis may be myopic. The clinical condition of CN, also referred to as bile cast nephropathy, includes impaired renal function in jaundiced patients along with characteristic histomorphological changes including intratubular cast formation and tubular epithelial cell injury predominantly at distal nephron segments (3). Consequently, apart from obstructive cholestasis CN has been described in a variety of different liver diseases ranging from drug-induced cholestatic liver injury to fulminant hepatitis and finally also liver cirrhosis. Despite the fact that the causal link between jaundice and kidney injury was established already in 1922 by Haessler et al. who studied urine sediments of jaundiced humans and dogs (4), the underlying pathophysiologic mechanisms of cholemic nephropathy are not entirely clear, and general accepted diagnostic criteria are still missing.

The current presentation will therefore summarize (i) the present knowledge on clinical and morphological characteristics of CN, (ii) available preclinical models, (iii) potential pathomechanisms, and (iv) potential future diagnostic and therapeutic strategies for CN. We have modelled CN in long-term common bile duct ligated mice (5). Experimental findings in wild type and FXR knock-out mice (having a hydrophilic bile acid pool) suggest that bile acid-mediated collecting duct injury represents one major trigger for CN (5). In addition, we recently demonstrated that treatment of common bile duct ligated mice with *nor*UDCA significantly ameliorated CN (6). These experimental findings point towards a pivotal pathophysiological role for bile acids in CN. Future clinical research in CN should clarify its prevalence in patients with liver diseases (e.g. advanced liver cirrhosis, ASH, PBC, PSC, bile duct atresia), focus on development of readily available biomarkers (e.g. urine cytology analysis, liquid biopsy), and overcoming the diagnostic dilemma of the need for invasive kidney biopsy by defining novel diagnostic criteria.

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**Figure 1:**



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## How the biliary tree maintains immune tolerance?

Xiong Ma\*<sup>1</sup>

<sup>1</sup>*Department of Gastroenterology and Hepatology, Renji Hospital, Shanghai Jiao Tong University, Shanghai, China*

**Corresponding author's email:** maxiongmd@163.com

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The liver is considered as a lymphoid organ with unique features of immune tolerance, consisting in clone deletion, anergy and unresponsiveness of various immune cells. Immune cells such as regulatory T cells, myeloid derived suppressive cells, NK cells, NKT cells, and  $\gamma\delta$ T cells play important roles to liver immune tolerance. The hepatic antigen-presenting cells (APCs) induce liver immune tolerance by down-regulating MHC class molecules and co-stimulatory molecules, while up-regulating co-inhibitory receptor ligands and secreting immunosuppressive cytokines. Interestingly, biliary epithelial cells (BECs) act as unconventional APCs which express MHC class II and costimulatory molecules, such as CD80 and CD86. In the other hand, BECs also recognize PAMPs of infectious agents by membrane Toll-like receptors, and produce chemokines and cytokines, resulting in the activation of the innate and adaptive immunity. Therefore, the regulatory activities of BECs are critical for the maintenance of peripheral immune tolerance in hepatic microenvironment. Primary biliary cholangitis (PBC) serves an example of a breakdown in central or peripheral immune tolerance to BECs. Molecular mimicry, genetic susceptibility and an imbalance in the immune microenvironment are also involved in the pathogenesis of autoimmune cholangitis. Restoration of the immune tolerance to BECs can be used as therapeutic strategies in various autoimmune cholangitis such as PBC.

# Bile acid homeostasis and immunity of the liver

**Christoph Schramm**<sup>\*1</sup>

<sup>1</sup>*1st Dept of Medicine, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany*


**Corresponding author's email:** cschramm@uke.de

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The liver has evolved to counteract toxic and immune mediated damage to protect the organ itself and the whole organism. This includes the strong tolerogenic function of the liver via multiple mechanisms. However, the liver synthesizes lipid metabolites such as bile acids to influence cellular functions in distant organs as well. In this context, lipid metabolites and specifically bile acids have long been recognized to influence immune cell function. On the other hand, systemic and hepatic inflammation are involved in the regulation of bile acid homeostasis, as observed in sepsis.

Hydrophobic bile acids contribute to cholestatic liver damage, in part by direct toxic effects on parenchymal cells. However, bile acids may also affect classical antigen presenting cells such as macrophages and dendritic cells, e.g. by promoting a pro-inflammatory phenotype by the modulation of inflammasome activation. Data elucidating the mechanisms involved in the modulation of immune cell function by bile acids are scarce and in part controversial, but most studies show an anti-inflammatory effect in part mediated by TGR5 and the nuclear receptor FXR. UDCA has been shown to inhibit the effect of TNF on monocytes in vitro and demonstrated immunomodulatory effects on hepatic cytokine expression in vivo. Significant effects on liver inflammation and on lymphocyte activation and proliferation were recently reported also for norUDCA in a murine Schistosomiasis model.

Ligands for bile acid receptors are currently being explored for the treatment of cholestatic liver diseases and act on many cell types. Of interest, obeticholic acid, a potent FXR agonist, has recently been shown to improve experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis which is mediated by T cells. Obeticholic acid had profound effects on T cell function. These results demonstrate, that bile acids modulate immune cell function far beyond the liver. These effects may be through direct action on effector cells, via antigen presenting cells or indirectly via effects on gut microbiota, which itself could induce metabolic changes modulating immune cell function at distant organs. In cholestasis, hepatocytes will downregulate bile acid synthesis and uptake and increase export via BSEP in order to reduce cell injury. In sepsis, elevated serum levels of bile acids can be used as prognostic markers indicating the degree of organ damage and predicting survival. We could recently show that CD8<sup>+</sup> T cell damage to the liver in a murine cholangitis model is by itself sufficient to induce adaptive changes in bile acid homeostasis. In summary, there is a complex and as yet incompletely understood interplay of bile acid



homeostasis and immune function. Understanding this interaction will be important to fully appreciate the value of bile acid derivatives and agonists of nuclear receptors for the treatment of cholestatic and inflammatory diseases of the liver and beyond.

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# IgG4 cholangiopathy, a new entity

Ulrich Beuers\*<sup>1</sup>, Lowiek Hubers<sup>1</sup>

<sup>1</sup>Academic Medical Center, Amsterdam, The Netherlands

**Corresponding author's email:** u.h.beuers@amc.uva.nl

IgG4-related disease (IgG4-RD) is a systemic inflammatory disorder with major manifestations in the pancreas in the form of autoimmune pancreatitis type 1 (AIP) and in the biliary tract and liver as IgG4 cholangiopathy [or IgG4-associated cholangitis (IAC)], often associated with elevation of serum IgG4 levels, infiltration of IgG4+ plasma cells in the affected tissue and good response to immunosuppressive treatment<sup>1</sup>. The first description of IgG4-RD of the bile ducts and pancreas may go back 150 years. The clinical presentation of its major hepatobiliary manifestation, IAC, may mimic other biliary diseases such as primary sclerosing cholangitis (PSC), various forms of secondary sclerosing cholangitis or cholangiocarcinoma. The HISORt criteria - Histopathology, Imaging, Serology [sIgG4], other Organ manifestations of IgG4-RD and Response to treatment - are regarded as a standard for the diagnosis of IAC. In this presentation, recent pathophysiological, diagnostic and therapeutic findings on IAC/AIP including own observations on (i) identification of highly specific, dominant IgG4+ B-cell receptor clones by advanced next generation sequencing (NGS) serving as a highly accurate diagnostic marker to distinguish IgG4-RD from PSC and biliary/pancreatic malignancies and possibly a key for better understanding the pathogenesis of IgG4-RD<sup>2,3</sup>, (ii) development and validation of blood IgG4-RNA/IgG-RNA qPCR as an affordable, highly accurate diagnostic marker comparable to NGS and possibly a future diagnostic standard for distinguishing IgG4-RD from PSC and biliary/pancreatic malignancies<sup>3</sup>; and (iii) 'blue collar work' with long-term exposure to solvents, paints, oil products or industrial gases as potential risk factors for development of IgG4-RD<sup>3,4</sup> are discussed. These and other recent findings may contribute to the understanding of the pathophysiology and to early diagnosis and adequate treatment of the so far enigmatic IgG4 cholangiopathy.

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## Target molecules in cholangiocarcinoma cells

Gregory J. Gores\*<sup>1</sup>

<sup>1</sup>*Gastroenterology and Hepatology, Mayo Clinic, Rochester, United States*

**Corresponding author's email:** gores.gregory@mayo.edu

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Cholangiocarcinoma (CCA) is a highly lethal hepatobiliary malignancy with limited therapeutic options. Advances in cholangiocarcinoma therapy will require an understanding of oncogenic signaling networks contributing to cholangiocarcinoma pathogenesis and which can be therapeutically disrupted. Promising driver mutations have potentially been identified in cholangiocarcinoma. Approximately 15% of cholangiocarcinomas are characterized by fibroblast growth factor receptor 2 (FGFR2) fusion gene aberrations. These FGFR2 fusion genes result in a protein which is constitutively active thereby driving oncogenic signaling networks. A variety of FGFR inhibitors are currently in human trials. One of these inhibitors, BGJ398 demonstrated a therapeutic efficacy signal in a small phase 2 trial. The other likely driver mutation is the IDH1 and 2 mutations present in 15-20% of cholangiocarcinomas. These IDH mutations result in generation of the hydroxyglutarate rather than alpha-ketoglutarate. This oncometabolite results in methylation changes in the DNA genome, thereby promoting carcinogenesis. There are trials using IDH inhibitors in human cholangiocarcinoma with IDH mutations; the one study which has been launched employs the small molecule inhibitor AG-120. The ultimate utility of this approach in treating cholangiocarcinoma remains to be determined. Another oncogenic pathway activated in cholangiocarcinoma is overexpression of MCL1. MCL1 is a prosurvival protein for human cholangiocarcinoma. Inhibition of its prosurvival effects and/or approaches to diminish protein expression may well be therapeutic in this cancer. Finally, there is a great deal of enthusiasm regarding immunotherapy for all solid tumours. Preliminary data using a PD-1 inhibitor suggests that a subset of patients will have a benefit which can be sustained. Additional data in humans will be necessary. We also note that the concept of combing cytotoxic therapy with immunotherapy is being tested in variety of human cancers. In our opinion, such therapies will be predicated on the fact that the additional compounds may induce or promote immunogenicity or immunogenic cell death. All of these concepts will be discussed during the presentation.

# Stem cell origin of cholangiocarcinoma

Tania Roskams\*<sup>1</sup>

<sup>1</sup>*Department of Translational Cell and Tissue Research, University of Leuven, Leuven, Belgium*

**Corresponding author's email:** [tania.roskams@uz.kuleuven.ac.be](mailto:tania.roskams@uz.kuleuven.ac.be)

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In contrast to continuously renewing systems like the hematopoietic system or the epidermis, the liver is a silent organ in which several cell types possess longevity: hepatocytes, cholangiocytes and progenitor cells. This implies that in the liver, several cell types can be targets during carcinogenesis. Hepatic progenitor cells are immature epithelial cells that reside in the smallest ramifications of the biliary tree in human liver. These cells are capable of differentiating towards the biliary and the hepatocytic lineage and represent the human counterpart of the oval cells in rodent liver. An increased number of progenitor cells (referred to as 'activation') and differentiation of the same towards hepatocytes (and/or bile duct epithelial cells) is a component of virtually all human liver diseases. Animal data indicate that oval cells are activated when the regenerative capacity of mature hepatocytes is inhibited, e.g. in response to necrogenic doses of carbon tetrachloride or due to oxidative stress, making these cells very likely carcinogenic targets. Direct evidence for the involvement of oval cells (progenitor cells) in the etiology of HCC was given by Dumble et al. (2002) who demonstrated that transplantation of oval cells derived from p53 knock-out mice into athymic nude mice gives rise to HCC.

In humans, chronic viral hepatitis B and C, mutagens like aflatoxin, metabolic diseases and alcoholic and non-alcoholic steatohepatitis are the most important risk factors for the development of HCC. Chronic inflammatory biliary diseases like hepatolithiasis (gall stones) are known risk factors for the formation of cholangiocarcinomas. This underscores that oxidative stress and chronic inflammation form common carcinogenic risk factors in all primary liver cancers. Under these conditions there is inhibition of hepatocyte proliferation, but activation of progenitor cells is favoured. Wiemann and coworkers (Wiemann et al., 2002) illustrated that, indeed, hepatocyte telomere shortening and senescence are general markers of human liver cirrhosis.

In humans, the two primary liver cancers are hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC). In addition, mixed forms of HCC and CC are described. Several recent detailed immunohistochemical studies have shown that e.g. HCC show a range of hepatocellular, biliary but also progenitor cell features, suggesting that at least part of the HCCs originate from progenitor cells. As one potential marker for progenitor cells, the expression of keratin 19 (CK19) has been identified. HCCs with progenitor features, i.e. expressing CK19, have a faster and higher recurrence rate after surgical treatment and CK19 overexpression correlates with HCC metastasis. Especially the prognostic value of CK19 in primary liver carcinoma has been extensively documented with CK19 expression being associated with a poor prognosis. Its validity was proven in

several studies by diverse methodologies, in different centres and in different ethnic patient groups. On the other hand, also in the spectrum of primary cholangiocarcinoma, mixed forms have been described, primarily seen as intrahepatic cholangiocarcinoma and in the context of chronic hepatitis. Classical hilar types of cholangiocarcinomas are seen in the setting of underlying biliary diseases like primary sclerosing cholangitis. These subtypes of cholangiocarcinomas have different kras mutation profiles. The side population, an isolation technique to enrich cancer stem cells, based on transporter expression of the cells, is higher in mixed tumours than in classical HCCs. The side population also correlates with chemoresistance.

How to distinguish ICC with mixed features from HCC with mixed features will be explained. It is clear that these subtypes are part of a spectrum of mixed forms of tumours with similar clinical behaviour.

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# Chemoresistance and chemosensitization in cholangiocarcinoma

Jose J.G. J. Marin\*<sup>1</sup>

<sup>1</sup>*Physiology and Pharmacology, HEVEFARM, University of Salamanca, IBSAL, CIBERehd, Salamanca, Spain*

**Corresponding author's email:** [jjgmarin@usal.es](mailto:jjgmarin@usal.es)

One major problem in the management of advanced cholangiocarcinoma (CCA) is the absence of effective treatment for these patients. None of the available anticancer drugs is significantly active against this tumour. A complex group of interacting processes determines the high refractoriness of CCA to pharmacological treatment [1]. Among these mechanisms of chemoresistance (MOC), the reduced intracellular concentration of active anticancer agents plays an important role. This can be the result of impaired drug uptake (MOC-1a), enhanced drug efflux (MOC-1b) or intracellular changes in the ability to either activate prodrugs or inactivate drugs (MOC-2). Other MOC, such as changes in molecular targets (MOC-3), DNA repairing capacity (MOC-4), altered survival/apoptosis balance (MOC-5), adaptation of cancer cells to tumour microenvironment (MOC-6) and the appearance of phenotypic transition (MOC-7), are also involved in the characteristic multidrug resistance (MDR) phenotype of liver cancer. In a previous study carried out on biopsies of hepatocellular carcinoma (HCC), hepatoblastoma and CCA the expression of approximately one hundred genes involved in MOC was analyzed [2]. We have observed that liver tumours have type-specific MOC-related genetic profiles together with threats that are shared among these types of tumors. Some of them involving genes included in MOC-1a and MOC-1b. A common feature is the marked reduction in the expression of OCT1 (gene symbol *SLC22A1*), a plasma membrane transporter playing a major role in the liver uptake of organic cations [3]. Interestingly, many inhibitors of tyrosine kinase receptors, such as sorafenib, are cations and are taken up by liver cancer cells through OCT1. This is a key step because the site of action of sorafenib is located intracellularly. The lack of OCT1 at the plasma membrane of tumour cells precludes a positive response to sorafenib in patients with HCC [4]. Recent studies suggest that aberrant splicing and epigenetic factors, such as enhanced promoter methylation, determine the low expression of OCT1 in CCA, which results in reduced drug uptake and poor pharmacological effect of sorafenib [5]. On the other hand, enhanced activity of ATP-binding cassette (ABC) export pumps, such as ABCB1, ABCG2 and some members of the ABCC family, results in additional lowering of intracellular concentrations of anticancer drugs. The expression of these ABC proteins, which is usually high in cancer cells, is further stimulated in response to pharmacological treatment. These characteristics constitute an important limitation for chemotherapy and offer valuable information that points towards the

molecular targets to be considered in the development of chemosensitizing strategies. Thus, we have developed chimeric constructs to be used in combination with appropriate viral vectors to induce in cancer cells the expression of drug uptake transporters under the control of ABC gene promoters. Preclinical phase studies have demonstrated that these constructs constitute efficient tools for chemosensitizing gene therapy, by enhancing anticancer drug concentrations in tumour cells. The stimulation of these promoters, which occurs spontaneously in chemoresistant cancer cells and can be further potentiated by pharmacological agents, such as dexamethasone, results in increased expression of the transgene, in this case the selected plasma membrane transporter. Consequently, drug uptake is improved, which leads to enhanced sensitivity to chemotherapy, as has been demonstrated in both *in vitro* and *in vivo* models [6]. In conclusion, a better understanding of MOC in CCA is required for the design of novel tools of gene therapy based on chemosensitizing transgenes under the control of specific and pharmacologically activatable promoters of MOC genes already upregulated in CCA.

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# Tumour stroma in cholangiocarcinoma: A novel therapeutic target

Joachim C. Mertens\*<sup>1</sup>

<sup>1</sup>University Hospital Zurich, Zurich, Switzerland

Corresponding author's email: [j.mertens@gmx.org](mailto:j.mertens@gmx.org)

**Difficulties in directly targeting tumour cells.** Specific therapeutic targeting of cancer cells has been the primary goal of oncological research for decades. However, effective cancer cell directed therapy has proven difficult to achieve mainly due to the enormous multitude of genetic aberrations in many types of cancer. This is particularly true for cholangiocarcinoma (CCC) where no targeted therapies exist today [1].

**Tumour stroma as important factor in tumour biology – the tumour ‘ecosystem’.** Malignant cells exist within a complex ‘ecosystem’ [2] consisting of a variety of cell types. Stromal cells of the tumour microenvironment have been shown to play a crucial role in tumour development, growth and metastasis. An important cell population aside from the malignant cells themselves are activated,  $\alpha$ -smooth muscle actin positive fibroblasts of the tumour microenvironment. These cells, also called cancer associated fibroblasts or CAF provide growth factors, modify and secrete extracellular matrix and facilitate cancer cell survival and metastatic mobility [3]. Of the multitude of interactions between tumour and stromal cells, the secretion of PDGF and the extracellular matrix components tenascin C and periostin by CAF have been demonstrated to promote tumour growth and metastasis [4]. Also, the number of CAF in the tumour microenvironment of cholangiocarcinoma patients is inversely correlated with patient survival. Thus, CAF are a potential therapeutic target in CCC.

**Perspectives of combined therapeutic approaches.** As effective and specific therapies targeting only the malignant cell are still a distant prospect in many cancers, combination cancer therapies, targeting CAF and the cancer cell may be the more promising and realistic therapeutic objective. In this multimodal approach, the tumour stroma offers a more uniform, less genetically heterogeneous target that should be a focus of future therapeutic developments. Exploiting the apoptotic priming of tumour stromal cells combined with other tumour cell targeted therapies could be an elegant approach for the treatment of desmoplastic cancers.

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## Cholangiopathies in children

**Richard J. Thompson**\*<sup>1</sup>

<sup>1</sup>*Institute of Liver Studies, King's College London, London, United Kingdom*

**Corresponding author's email:** richard.j.thompson@kcl.ac.uk

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That bile ducts are susceptible to damage through a variety of mechanisms, is not news to this audience. In children, the spectrum is however somewhat different from older individuals. These differences will be the focus of this presentation. Genetic and autoimmune mechanisms are frequent, but those of complex aetiology predominate.

Biliary atresia (BA) is the most frequent underlying indication for liver transplantation in children. It represents an intense inflammatory sclerosing cholangiopathy, causing complete occlusion of at least part of the biliary tree. BA should really be thought of as a phenotype rather than as a disease. In only extremely rare cases is there any familial occurrence. The incidence varies globally from 1/15,000 to 1/8,000. Viral infection has been associated with BA, most recently CMV, but there is no evidence of pathogens in the majority. Autoreactive antibodies have been described in some patients, but the lack of familial recurrence argues against an allo-immune process. Whatever the predisposing factors, the patients all present in the first few months of life. Bypassing the occluded extrahepatic biliary tree with a Roux-en-Y loop, at Kasai portoenterostomy, has changed the natural history, though is not curative. Anti-inflammatory treatment, such as steroids, have so far proved ineffective.

Primary sclerosing cholangitis, per se, is rare in children. However, a very similar phenotype is usually seen in the context of autoimmune liver disease. This probably corresponds to the AIH/PSC overlap syndrome seen in adults but, because evident autoimmunity is the norm, is often referred to as autoimmune sclerosing cholangitis. The majority have inflammatory bowel disease, though not always clinically manifest.

The biliary tree is prone to opportunistic infections in the face of immune deficiency, and the resulting cholangiopathy can lead to end stage liver disease requiring combined liver and haematopoietic stem cell transplant. The most common infections are *Pneumocystis* and *Cryptosporidium*.

Langerhan's cell histiocytosis is an unusual condition of childhood. Although the disease remits, the mortality remains significant, as a result of target organ damage. The liver is thankfully an infrequently affected organ, but the biliary tree is the target.

MDR3 deficiency is confusingly thought of as a form of progressive familial intrahepatic cholestasis. However, the reduced phospholipids in bile mean higher concentrations of monomeric bile acids and result in a cholangiopathy. Precipitates of cholesterol are sometimes manifest, even in the smallest bile ducts.

Neonatal sclerosing cholangitis, as the name suggests, is condition particular to early childhood. Clinically and histologically it looks very similar to biliary atresia, though with patency of the biliary tree. Most patients do not have extrahepatic disease, it is however enriched in consanguineous families. We recently described mutations in DCDC2, in a subset of such patients. The gene encodes an intraflagellar transport complex protein, and the disease in these patients is therefore a ciliopathy.

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## Genetics of familial cholangiopathies

**Frank Lammert**<sup>\*1</sup>

<sup>1</sup>*Department of Medicine II, Saarland University Hospital, Homburg, Germany*

**Corresponding author's email:** frank.lammert@uks.eu

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Familial cholangiopathies are rare but potentially severe diseases. Their spectrum ranges from rather benign disorders as, for example, recurrent intrahepatic cholestasis (BRIC) and low-phospholipid associated cholelithiasis (LPAC) to progressive familial intrahepatic cholestasis (PFIC). Familial cholangiopathies affect first and early bile duct function but some of them, like for example PFIC, might also cause progressive damage to the liver parenchyma and result in end-stage liver disease.

In recent years, our understanding of these cholestatic diseases has improved, since we have been able to pinpoint numerous disease-causing mutations that cause familial cholangiopathies. Given the availability of genotyping resources, these findings can be introduced in the diagnostic work-up of our patients. Moreover, functional studies have allowed the mechanistic dissection of the pathophysiological consequences of the detected variants. In the future, these findings will contribute to new therapeutic strategies for patients with familial cholangiopathies.

In this review, we present the latest data on the genetic background of familial cholangiopathies and discuss their application in clinical practice for the differential diagnosis of cholestasis of unknown aetiology. As look in the future we will introduce system genetics as a novel experimental tool of investigating cholangiopathies and their modifiers.

# Cholangiocarcinoma: Epidemiological trends and clinical management

**Shahid A. Khan**\*<sup>1</sup>

*<sup>1</sup>Imperial College Healthcare NHS Trust /Imperial College London, London, United Kingdom*

**Corresponding author's email:** shahid.khan@imperial.ac.uk

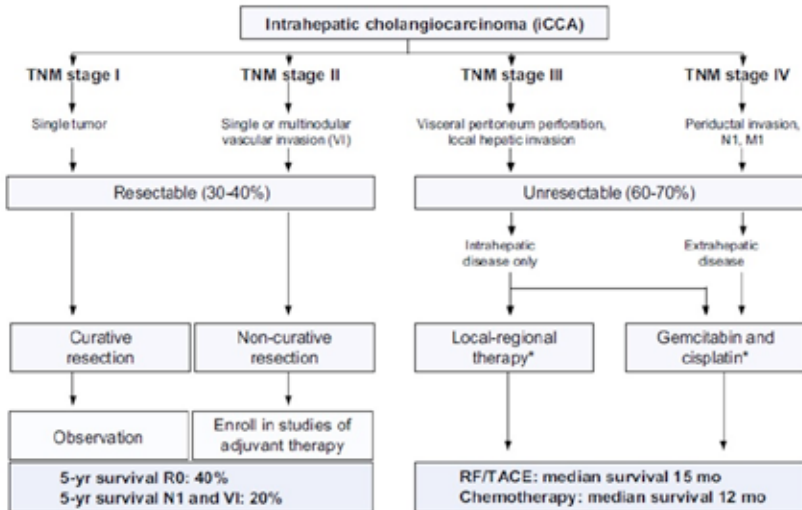
Cholangiocarcinoma (CCA) is an adenocarcinoma arising in the biliary tree and represent 3% of all gastrointestinal malignancies. CCA has a peak age incidence in the seventh decade and occurs in both genders, albeit with a slight male preponderance. CCA can be sub-divided into intrahepatic, peri-hilar or extra-hepatic. Overall, it is estimated that approximately 50% of CCA are perihilar (pCCA), arising at bifurcation of the main hepatic ducts; 20-30% arise in the distal extrahepatic bile ducts (eCCA) and 20-30% arise within the intrahepatic bile ducts of liver (iCCA). Overall, intrahepatic CCA represent 5-10% all primary liver cancers. These three sub-groups differ in their respective their pathogenesis, risk factors, epidemiological trends, clinical presentations, diagnostic modalities and treatments.

The global prevalence of CCA varies significantly throughout the world, likely reflecting differences in geographical risk factors as well as genetic differences. The highest rates are in North East Thailand. Recognised risk factors for CCA include Parasitic Infection (*Opisthorcis viverrini*, *Clonorchis sinensis*), Primary sclerosing cholangitis, Fibropolycystic Liver Disease, Intrahepatic Biliary Stones, Chemical Carcinogen Exposure, Chronic Liver Disease, Viral Hepatitis, Obesity and Type 2 Diabetes. Any potential genetic predisposition to CCA is currently poorly understood. There have been several published studies examining epidemiological trends in liver cancer incidence and mortality rates, including for CCA. Overall recent trends in most countries are similar for both hepatocellular carcinoma and CCA, on an upward trajectory. Although CCA rates overall seem to be increasing, when examining sub-types, rates for iCCA seem to be increasing and rates for eCCA appear to be declining. However, data from Thailand, France, and Italy show that in those countries iCCA rates have increased whilst those for HCC have decreased. In Japan however, rates for both HCC and CCA seem to be declining in recent studies. Although HCC and iCCA are now recognised to harbour common risk factors, geographic areas of increasing iCCA rates do not entirely correspond with those of increasing HCC rates. The accuracy of these studies may be affected by the quality of data as CCA can be difficult to diagnose, there are no accurate tumour markers, imaging is not always definitive and histology not always available. Furthermore, changes to ICD coding may have played a role in the fluctuations of the sub-types of CCA.

The clinical management of CCA is challenging. The disease carries a high mortality and overall five-year survival is less than 10%. The reasons are that most patients have no

recognised risk factors and thus develop sporadic disease. There is no proven screening method to detect this cancer and most patients present with clinical symptoms, by which time the underlying cancer not amenable to curative resection, which is the only chance of cure. Liver transplantation is rarely done in most countries due to historic high recurrence of the cancer in grafts, although some US centres have reported excellent outcomes in carefully selected patients. Treatment options depend on the stage at presentation (Figure 1) and the patient's general performance status, including co-morbidities. Published studies suggest that, overall, Surgical Resection (R0) offers a five-year survival of up to 40%, depending on the centre's expertise. With local regional therapies such as ablation, overall survival varies from around 20 to 30 months; with Transarterial therapies up to 24 months and cytotoxics on average up to 12 months, with the typical regime being combination therapy with Gemcitabine and Cisplatin chemotherapy. Biliary stenting, for palliation or pre-surgical drainage is also commonly employed. This lecture will present an overview of recent global epidemiological trends of CCA rates and summarise the clinical management.

### ILCA Guidelines on iCCA, *J Hepatology*



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# Assessment of hepatic secretion function with IIC-cholylsarcosine PET/CT

Michael Soerensen\*<sup>1,2</sup>

<sup>1</sup>Dept. of Hepatology and Gastroenterology, <sup>2</sup>Dept. of Nuclear Medicine and PET Centre, Aarhus University Hospital, Aarhus, Denmark

**Corresponding author's email:** michsoer@rm.dk

The conjugated bile acid analogue [*N*-methyl-<sup>11</sup>C]cholylsarcosine (<sup>11</sup>C-CSar) has been developed as a tracer for functional positron emission tomography (PET) of the hepato-biliary secretion. <sup>11</sup>C-CSar PET represents an extraordinary possibility for *in vivo* quantification of the individual transport steps involved in hepato-biliary secretion of conjugated bile acids and was validated for this purpose in pigs [1] and patients with cholestatic disorders of different origin and healthy subjects [2]. The integrity of the transport steps involved in hepatic uptake of bile acids from blood and subsequent secretion into bile is crucial for preventing accumulation of toxic amounts of bile acids in hepatocytes and in the systemic circulation and <sup>11</sup>C-CSar PET enables a new characterization of the pathophysiological features of cholestatic disorders [2]. For example, in patients with alcohol liver disease, but not in patients with primary biliary cholangitis or primary sclerosing cholangitis, the permeability surface area product for the transport from blood to hepatocytes ( $PS_{mem}$ ) was decreased when compared to healthy subjects. Moreover, in all types of cholestatic disorders studied so far, the rate constant for secretion from hepatocytes to bile was decreased; the mean residence time for <sup>11</sup>C-CSar in hepatocytes was significantly prolonged in spite of increased backflux of tracer from hepatocytes to blood [2]. Such pathophysiological insight cannot be obtained using other methodologies. <sup>11</sup>C-CSar PET also allows for direct evaluation of the potential effect of new drugs on the transporter steps involved in hepato-biliary secretion of conjugated bile acids.

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# The future of therapy for cholangiopathies

**Michael Trauner**<sup>\*1</sup>

*<sup>1</sup>Internal Medicine, Gastroenterology and Hepatology, Medical University of Vienna, Vienna, Austria*

**Corresponding author's email:** michael.trauner@meduniwien.ac.at

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Since the etiology and pathogenesis of PBC and PSC are still poorly understood and unlikely to be resolved in the near future, our current therapeutic options are still rather limited. Sometimes secondary (potentially treatable) causes of sclerosing cholangitis (SSC) can be identified, which may apply to PSC (and PBC). Irrespective of the cause of cholangiopathies, several therapeutic strategies aim at restoring impaired bile secretion and reducing biliary toxicity. This may be a main mode of action of ursodeoxycholic acid (UDCA) as paradigm therapeutic bile acid (BA) and first-line treatment for PBC. However, its therapeutic efficacy appears limited and is still under debate in PSC, and PBC patients with insufficient biochemical response to UDCA still require additional therapies.

Novel drug targets for the future include the BA receptors FXR (as well as other nuclear receptors) and TGR5, BA-induced gut hormones FGF19 and GLP-1, and BA transport systems such as ASBT and Ntcp within the enterohepatic BA circulation. As key regulator of BA homeostasis with additional anti-inflammatory and immunomodulatory properties, FXR represents an attractive therapeutic target for cholestatic liver diseases such PBC and PSC. Preclinical evidence suggests, that targeting FXR may not only reverse the underlying cholestatic abnormalities, but also counteracts hepatic (as well as intestinal) inflammation, the progression to liver cirrhosis and cancer, as well as complications of portal hypertension with intestinal bacterial translocation. The first in class steroidal FXR ligand obeticholic acid (OCA) improved cholestasis and inflammatory markers in PBC patients, but induced pruritus in a dose-dependent fashion. OCA is currently also tested in PSC. Non-steroidal FXR ligands (currently undergoing phase II trials in PBC and PSC) may have different pharmacokinetic and therapeutic / side effect profiles. Targeting the intestinal FXR-FGF19-axis appears attractive, since several therapeutic FXR effects could be explained by changes in FGF19 signaling as well as gut inflammation and microbiota. Modulation of BA transport within the enterohepatic circulation may also modify BA signaling along their enterohepatic circulation. Interestingly, opposing strategies targeting FXR-FGF19 (stimulation via FXR ligands or exogenous FGF19 mimetics vs. inhibition with ASBT blockers or resins) may be beneficial in cholestasis. Additional approaches may target other nuclear receptors such as RAR, VDR, and PPARs which are also involved in regulating biliary homeostasis and inflammation. Of note, several smaller studies have tested fibrates (ligands for PPARα) in PBC (and PSC); controlled clinical studies are

eagerly awaited. Other ligands for a/d (elafibranor) and PPAR $\delta$  (MBX-8025) are also tested in PBC.

Another promising novel BA-based therapeutic strategy includes 24-*nor*ursodeoxycholic acid (*nor*UDCA), a side chain-shortened C<sub>23</sub> homologue of UDCA, undergoing cholehepatic shunting with direct anti-inflammatory and anti-fibrotic actions. Recently, *nor*UDCA has been shown to improve cholestasis in a phase II study in PSC. Notably these results were independent from previous exposure and response to UDCA and the drug was well tolerated without inducing pruritus.

Although immunomodulatory/immunosuppressive therapeutic strategies have so far been rather disappointing in PBC and PSC, the potential of newer biologic agents deserves future evaluation. These include gut-specific  $\alpha 4\beta 7$ -integrin-neutralising monoclonal antibodies (e.g., vedolizumab) or anti-VAP-1 (responsible for inducing expression of MAdCAM-1 thereby driving hepatic inflammation/fibrosis) in PSC or anti-CD40 (triggering activation of NF- $\kappa$ B and AP-1, leading to bile duct loss / apoptosis) in PBC. The tyrosine kinase/janus kinase inhibitor tofacitinib has beneficial effects in ulcerative colitis and targets pathways which are also involved in PSC. Directly inhibiting fibrosis remains an attractive target, but the results obtained with simtuzumab, a monoclonal antibody against lysyl oxidase homolog 2, did not show clinical efficacy in PSC. In view of the possibility that biliary infection or composition of the gut microbiota may contribute to PSC pathogenesis, the role of antibiotics (and pre-/pro-biotics) deserves further evaluation. Importantly, nuclear receptor (e.g., FXR, PPAR) ligands may also act as immuno-metabolic drugs which not only impact on cholestasis, but they also modulate primary or secondary immunological and inflammatory (epi)phenomena in cholestasis. After a long period of rather limited pharmacological options, a future challenge will be to clinically test the exploding number of therapeutic strategies and their potential combinations. Very likely, complex and heterogeneous disorders such as PBC and PSC will require combination therapies and/or personalized therapies tailored to subtype and stage of the disease in the future.

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## Cholestatic drug induced liver injury

**Raul J. Andrade**<sup>\*1,2</sup> and **Spanish DILI Registry**

<sup>1</sup>Medicine, University of Malaga, <sup>2</sup>Unidad de Gestión Clínica de Aparato Digestivo, Instituto de Investigación Biomédica de Málaga-IBIMA, Hospital Universitario Virgen de la Victoria. Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Málaga, Spain

**Corresponding author's email:** andrade@uma.es

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Idiosyncratic drug-induced liver injury (DILI) is a complex and multi-layered disorder that affects susceptible subjects exposed to therapeutic doses of drugs and whose pathogenesis is still poorly understood. Many drugs in common use and a variety of xenobiotics can cause toxic cholestasis. The bulk of cases are unpredictable idiosyncratic or hypersensitivity reactions. While the meaning of drug-induced cholestasis can vary depending on whether it is referred to the mechanistic, pathological or clinical level, for practical and investigative purposes toxic cholestasis is better defined as a particular phenotype, which by consensus is based in the activities of liver enzymes. Following these criteria, the cholestatic pattern is defined as a >2-fold elevation above the upper limit of normal (ULN) for alkaline phosphatase (ALP) or if alanine aminotransferase (ALT) levels are concomitantly raised a ratio  $ALT \times ULN / ALP \times ULN \leq 2$ . Toxic cholestasis as previously defined, ranks in frequency behind the hepatocellular type of liver damage being more common in elderly patients and as such leads more often to non-liver related mortality than the hepatocellular pattern. In rare instances cholestatic DILI may evolve to a chronic cholestatic syndrome resembling primary biliary cholangitis. Liver biopsy features of bile duct loss early in the outcome have been recently shown as a predictor for this worst outcome (Bonkovsky et al *Hepatology* 2017). Genetic variants that may contribute to susceptibility to drug-induced cholestasis are being identified. An ancillary study, using a candidate gene approach, already shown that HLA class II alleles were associated with either risk (DRB1\*15 and DQB1\*06) or protection (DQB1\*02) for cholestatic/mixed pattern of injury due to an array of drugs. Genome wide association studies (GWAS) performed in individuals with DILI (mostly cholestatic) related to flucoxacillin and amoxicillin-clavulanate showed strong association with an HLA class I allele (B\* 57:01) for flucoxacillin (Daly et al *Nat Gen* 2009) and HLA class II (DRB1\*15:01) for amoxicillin- clavulanate (Lucena et al *Gastroenterology* 2011). A new genetic association related to a relatively rare HLA class I allele (A\*33:01) has been recently identified and replicated in a large cohort of DILI cases due to a wide range of licensed drugs (Nicoletti et al *Gastroenterology* 2017). Interestingly, The A\*33:01 association was genome-wide significant only for cholestatic/mixed DILI (OR=5.2) and was driven by large effects in DILI due to terbinafine (OR=40.5), fenofibrate (OR=58.7) and ticlopidine (OR=163.1). The association of A\*33:01 with DILI in general and

secondary to a number of structurally dissimilar compounds is consistent with previous observations and together with recent findings from in vitro studies on T-cell responses to flucloxacillin and amoxicillin-clavulanate, support the hypothesis that either the parent drug or metabolites bind covalently to cellular or circulating proteins to form adducts. Adduct formation may then allow binding to the peptide binding groove of HLA molecules leading to activation and differentiation of T-cells with a consequent adaptive immune response-mediated liver injury. Evidence that the majority of the drugs showing the A\*33:01 association undergo hepatic metabolism and biliary excretion may explain the association of A\*33:01 with cholestatic pattern of DILI and could indicate that metabolites contribute to the toxicity mechanism. Nevertheless, no one genetic factor has shown power enough to predict toxic cholestasis in a given individual. The bile salt export pump (BSEP) inhibition can lead to impaired bile flow and cholestasis, as seen in patients with ABCB11 mutations and familial intrahepatic cholestasis. Besides, drug-induced BSEP inhibition has been linked to hepatotoxicity with studies demonstrating correlations between a drug's ability to inhibit BSEP in vitro and its known hepatotoxicity potential. Furthermore, BSEP inhibition has been reported to be associated with physicochemical drug properties, such that drugs with higher molecular weight and higher calculated LogP value are positively correlated with BSEP inhibition potency.



## Cell transplantation

**Domenico Alvaro**<sup>\*1</sup>

<sup>1</sup>*Internal Medicine and Medical Specialties, Sapienza University of Rome, Rome, Italy*

**Corresponding author's email:** domenico.alvaro@uniroma1.it

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Liver diseases represent a major public health problem affecting 5-15% of the inhabitants worldwide. Currently, orthotopic liver transplantation (OLT) is the only therapy for acute and chronic liver diseases in the terminal stage. However, the number of donated livers is limited. In addition, OLT cannot be proposed for many cirrhotic patients, since at very advanced disease stages, surgical risks or other contraindications exist. These limitations drive interests to explore cell therapies aimed to sustain liver function, to stimulate liver regeneration and to reduce the scarring process. In a series of published studies and summarized in recent reviews (1-3), we demonstrated the presence of cells expressing a variety of endodermal stem cell markers in (peri)-biliary glands (PBGs) of bile ducts in fetal to adult human tissue. We also found them in crypts of gallbladder epithelium. The observations *in situ* in human biliary tree tissue have been complemented by demonstrations of culture selection of colonies of stem cells and that expand for months through self-renewal processes, and are multipotent as indicated by their ability to lineage restrict to hepatocytes, cholangiocytes versus pancreatic islet cells depending on the precise conditions to which they are subjected. Isolation of major subpopulations of hBTSCs from tissues of all donor ages can be achieved by immunoselection for cells positive for expression of epithelial cell adhesion molecule (EpCAM). The hBTSC subpopulations are all small (7-9  $\mu\text{m}$ ), approximately half the size of mature parenchymal cells; express various stem cell markers such as endodermal transcription factors (SOX9, SOX17, PDX1), pluripotency genes (OCT4, SOX2, NANOG, SALL4, KLF4/5), CD133 (prominin), CD44 (hyaluronan receptors), aldehyde dehydrogenase (ALDH), and hedgehog proteins and no markers of mature hepatic or pancreatic cells. They are also tolerant of ischemia. Preliminary studies indicated that we can isolate viable and healthy hBTSCs from biliary tree tissue even 48 hours or more after cardiac arrest of the donor. In addition, other preliminary studies suggest that the hBTSCs cryopreserve under serum-free, wholly defined conditions that we established and found effective for human hepatic stem cells. In preclinical studies, we demonstrated that hBTSCs isolated from human gallbladder and transplanted in a model of liver cirrhosis yielded the formation of human hepatocytes and cholangiocytes and, mostly important, the improvement of the liver function tests in the model. The hBTSCs can be isolated from fetal or adult livers or from adult gallbladder. BTSCs is an ideal source of multipotent endodermal stem cells with relevant self-renewal and differentiative capacities. First, tissues are largely available since fetal biliary trees can be easily obtained from the fetal liver given the large number of therapeutic abortions, postnatal biliary tree tissues are largely available since most of the biliary tree is routinely

discarded with liver and pancreatic transplants and, gallbladder tissue is readily available given large numbers of cholecystectomy done routinely. Second, the hBTSCs can be isolated easily from biliary tree of any donor age and survive and expand *ex vivo* under wholly defined and serum-free conditions that we established; v) hBTSCs from fetal tissue are non- or minimally immunogenic given their low or null expression of HLA class I and II antigens and, therefore, can be transplanted without the need for immunosuppression; vi) hBTSCs, transplanted into experimental models of liver cirrhosis, are able to engraft, to differentiate into adult hepatocytes and cholangiocytes and to rescue liver function. The use of stem/progenitor cells has advantages over transplantation of mature hepatocytes. Firstly, stem/progenitor cells, capable of generating mature liver cells, can circumvent the shortage of hepatocytes. The hBTSCs are available from hepatic and biliary tree tissues of donors of all ages and can self-replicate under particular conditions indefinitely. Secondly, transplanting stem/progenitor cells may, in theory, yield better long-term repopulation and persistent metabolic activity due to the constant generation of newly formed mature parenchymal cells. Thirdly, stem/progenitor cells are not immunogenic, are more resistant than adult cells to ischemic damage, and are easier to cryopreserve. All of these properties enable stem/progenitors to be a more robust product for use in clinical programs. Indeed, in the first two cirrhotic patients treated in our centre with intra-arterial infusion of HBTSCs, we registered no adverse event but significant benefits.

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## Endpoints in the designing of clinical trials

Cyriel Ponsioen\*<sup>1</sup>

<sup>1</sup>*Gastroenterology and Hepatology, AMC, Amsterdam, The Netherlands*

**Corresponding author's email:** [c.y.ponsioen@amc.uva.nl](mailto:c.y.ponsioen@amc.uva.nl)

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In primary sclerosing cholangitis (PSC) there is an urgent need for defining endpoints for clinical trials. Firstly, there is currently no therapy that can halt disease progression. Secondly, there are at present no approved endpoints other than solid clinical endpoints. Thirdly, there is a resurgence of interest from pharma companies in this rare and dismal disease.

In regulatory terms, a clinical endpoint measures how a patient feels, functions or survives. In order to qualify for regular or full approval, biomarkers that measure benefit in any of these domains should be validated in clinical trials. Accelerated or conditional approval may be granted in serious or life threatening conditions, when benefit can be shown based on a biomarker that is a surrogate that is reasonably likely to predict clinical benefit. There are currently no validated endpoints in PSC for measuring cholestatic symptoms or disease-specific quality of life. When it comes to improving survival the design of clinical trials should rely on surrogate endpoints, because the occurrence of solid endpoints such as death and/or transplant are too infrequent and take too long to occur for any clinical trial to serve as feasible primary outcome.

Hence, it is of great importance to develop validated surrogate endpoints for this disease. The International PSC Study Group recently conducted a consensus process with the purpose to identify surrogate endpoints for clinical trials. This consensus process yielded a shortlist of five candidates as surrogate endpoints for measuring disease progression: alkaline phosphatase, transient elastography, histology, a combination of alkaline phosphatase + histology, and bilirubin. Histology, alkaline phosphatase, and transient elastography came out as most promising. However, the expert panel stated that no biomarker currently exceeds level 3 validation. To compensate for this uncertainty, it was advised to combine endpoints.

This presentation will cover the most likely candidate surrogate endpoints for measuring disease progression, as well as the concerted actions that are necessary by all stakeholders to promote validation of these.

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## Principles of value based medicine as applied to cholangiopathies

### Mario Strazzabosco\*<sup>1</sup> on behalf of The Value Based Medicine in Hepatology Study and The value-based Medicine in Hepatology Study Group

<sup>1</sup>Liver Center- Internal Medicine, Yale University, New Haven, CT, United States

**Corresponding author's email:** [mario.strazzabosco@yale.edu](mailto:mario.strazzabosco@yale.edu)

Chronic cholangiopathies impose a major health burden for patients, their families and for their health care system. Their treatment requires multi-disciplinary coordination, labor-intensive support for critically ill patients and effective chronic disease management. The long natural history of these diseases and their relatively low prevalence complicate the analysis of treatment efficacy and outcomes. These are required to re-orient competition and decision making around the principle of maximizing the value of care for the patient. A comprehensive set of OIs for liver diseases is not currently available. The Value-based Medicine in Hepatology (VBMH) study aims to fill this gap, devising and testing a set of outcome indicators for major liver conditions. Here we will present an interim analysis of the study (Value-based Medicine in Hepatology, VBMH) for the subgroup of patients with Primary Sclerosing Cholangitis. The VBMH study was performed in Italy between 2011 and 2013 and the follow-up still continues. Here we will report an interim analysis of the Primary sclerosing cholangitis (PSC) is a rare and progressive chronic cholangiopathy with scarce therapeutic leading to liver cirrhosis and/or malignancies like cholangiocarcinoma (CCA) or colorectal cancer (CRC). The clinical management of PSC remains challenging and in other rare or relatively rare diseases the availability of outcome indicators would be of extreme importance to guide treatment, decision making and allocation of resources. PSC, along with primary biliary cholangitis (PBC) were part of a study aiming at: A) identifying Clinical Outcome Indicators (COIs) for PSC, and B) validating OIs in a clinical context.

The Study included two phases. In phase A) A panel of experts generated a list of OIs using a modified version of the Delphi method. B) OIs with the highest RAND/UCLA score were tested in an ongoing multicentric and prospective study.

Five COIs were identified on the basis of the highest rating values (around 9) and the lowest disagreement indexes (next to 0). They include: annual rate of acute cholangitis episodes (OI#1); mortality rate for patients not yet listed for liver transplantation (OI#2); rate of quality of life improvement, measured by EQ-VAS (a visual assessment scale ranging from 0 to 100 where the patient points out his present-day health status) and EQ-5D (assessment of 5 domains that measure daily performance: mobility, selfcare, anxiety/depression, usual activities and pain/discomforts, according to 3 levels of severity)

(OI#3); number of patients died for CCA and CRC (OI#4); incidence and/or worsening of osteoporosis, expressed as T-score differential over a 2-year interval (OI#5).

In the validation study, 63 consecutive patients with PSC enrolled in 3 tertiary liver centres in the Northern Italy were evaluated for a 24-month follow-up period. For each OI, the following values were reported: OI#1) cumulative incidence of cholangitis was 5.2%, resulting in 0.029 cholangitis/patient; OI#2 and OI#4) no patients died without being listed for transplantation or because of cancer during the study time; OI#3) 38.9 and 19.4% of patients showed an improvement in EQ-VAS and EQ-5D parameters, respectively during follow-up; OI#5) 3% of patients developed or worsened osteoporosis. The five COIs for PSC were identified based on a highly shared consensus. Albeit the study population is small (as in the case of rare diseases, as PSC in Italy) and the follow-up time is short as compared to the long natural history of the disease, these OIs have proven to be easy to collect. Therefore, they are suitable to be extended to specialized centres involved in PSC management to further validate their clinical usefulness in a larger scale. However, in a disease marked by a long natural history such as PSC, the inclusion also of patient-reported outcomes, including quality of life, will probably become one of the major end points.

### **The Value Based Medicine in Hepatology Study Group**

Strazzabosco M<sup>1,3,7</sup>, Carbone M<sup>1,3,8</sup>, Ciaccio A<sup>1,3</sup>, Cristoferi L<sup>1</sup>, Okolicsanyi S<sup>1,3</sup>, Cozzolino P<sup>4</sup>, Rota M<sup>4</sup>, Cortesi PA<sup>1,4</sup>, Belli LS<sup>3,6</sup>, Fagioli S<sup>5</sup>, Cesana G<sup>4</sup>, Mantovani LG<sup>3,4</sup>, Fabris L<sup>2,3</sup>

1. Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy;
2. Department of Molecular Medicine, University of Padua, Padua, Italy;
3. International Center for Digestive Health, University of Milan-Bicocca, Milan, Italy
4. Research Centre on Public Health (CESP), University of Milan-Bicocca, Milan, Italy;
5. Department of Gastroenterology, Papa Giovanni XXIII Hospital, Bergamo;
6. Department of Hepatology and Gastroenterology, Liver Unit. Niguarda Hospital, Milan, Italy;
7. Liver Center and Section of Digestive Diseases, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA.

# ORAL ABSTRACT PRESENTATIONS

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## Serum extracellular vesicles contain protein biomarkers for primary sclerosing cholangitis and cholangiocarcinoma

Ander Arbelaiz<sup>1, 2</sup>, Mikel Azkargorta<sup>3, 4</sup>, Alvaro Santos-Laso<sup>5, 6</sup>, Maria J. Perugorria<sup>2, 4, 7, 8</sup>, Oihane Erice<sup>2, 7</sup>, Esperanza Gonzalez<sup>4, 9</sup>, Ainhoa Lapitz<sup>2, 7</sup>, Laura Izquierdo<sup>2, 7</sup>, Paula Olaizola<sup>2, 7</sup>, Pui Y. Lee-Law<sup>7</sup>, Ane Arregi<sup>2, 7</sup>, Raul Jimenez-Aguero<sup>2, 7</sup>, Adelaida Lacasta<sup>2, 7</sup>, Cesar Ibarra<sup>10</sup>, Alberto Sánchez-Campos<sup>10</sup>, Juan P. Jimeno<sup>11</sup>, Marcin Krawczyk<sup>12, 13</sup>, Frank Lammert<sup>12</sup>, Marco Marzioni<sup>14</sup>, Rocio I. Macias<sup>4, 15</sup>, Jose J. G. Marín<sup>4, 15</sup>, Tushar Patel<sup>16</sup>, Gregory J. Gores<sup>17</sup>, Ibon Martinez<sup>18</sup>, Felix Elortza<sup>3, 4</sup>, Juan M. Falcón-Pérez<sup>4, 8, 9</sup>, Luis Bujanda<sup>4, 6, 7</sup>, Jesus Banales<sup>2, 4, 7, 8</sup>

<sup>1</sup>Department of Liver and Gastrointestinal Diseases, Biodonostia Research Institute, Donostia University Hospital, <sup>2</sup>University of the Basque Country (UPV/EHU), San Sebastián, <sup>3</sup>Proteomics Platform, CIC bioGUNE, Derio, <sup>4</sup>National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Carlos III National Institute of Health, Madrid, <sup>5</sup>Department of Liver and Gastrointestinal Diseases, Biodonostia Research Institute – Donostia University Hospital, <sup>6</sup>University of the Basque Country (UPV/EHU), San Sebastian, <sup>7</sup>Department of Liver and Gastrointestinal Diseases, Biodonostia Research Institute – Donostia University Hospital, San Sebastián, <sup>8</sup>Ikerbasque, Basque Foundation for Science, Bilbao, <sup>9</sup>Metabolomics Unit, CIC bioGUNE, Derio, <sup>10</sup>Cruces University Hospital, Bilbao, <sup>11</sup>Complejo Universitario de Navarra, Pamplona, Spain, <sup>12</sup>Department of Medicine II, Saarland University Medical Center, Saarland University, Homburg, Germany, <sup>13</sup>Laboratory of Metabolic Liver Diseases, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Warsaw, Poland, <sup>14</sup>Department of Gastroenterology, “Università Politecnica delle Marche”, Ancona, Italy, <sup>15</sup>Experimental Hepatology and Drug Targeting (HEVEFARM), Biomedical Research Institute of Salamanca (IBSAL), University of Salamanca, Salamanca, Spain, <sup>16</sup>Department of Cancer Biology, Mayo Clinic, Jacksonville, <sup>17</sup>Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, United States, <sup>18</sup>OWL Metabolomics, Derio, Spain

**Corresponding author's email:** [ander.arbelaiz@biodonostia.org](mailto:ander.arbelaiz@biodonostia.org)

**Introduction:** Cholangiocarcinoma (CCA) includes a heterogeneous group of biliary cancers with poor prognosis. Several conditions such as primary sclerosing cholangitis (PSC) are risk factors. Accurate non-invasive biomarkers for CCA or PSC are not available. In the last years, extracellular vesicles (EV) have emerged as an important tool in the search of biomarkers for different disorders as well as pathogenic players involved in disease development and progression.

**Aims:** To investigate the potential role of serum EV as carriers of protein biomarkers for PSC and CCA, as well as oncogenic proteins that might be involved in tumor growth and dissemination.

**Material and Methods:** Serum EV were isolated from CCA (n=13) or PSC (n=9) patients and healthy individuals (n=10) using well-established ultracentrifugation/filtration methods. In addition, EV were isolated from the culture medium of normal human cholangiocytes (NHC), SV-40 immortalized human cholangiocytes (H69) and two CCA human cell lines (i.e. EGI1 and TFK1). The characterization of EV was performed by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA, Nanosight) and immunoblot. The proteome of EV was determined by mass spectrometry-based proteomics.

**Results:** Serum EV concentrations were found higher in CCA and PSC than in controls by NTA (Nanosight). Round morphology (by TEM), size (~165 nm diameter by NTA) and markers (CD9, CD63 and CD81 by immunoblot) indicated that most serum EV were exosomes. Proteome profiles (by mass spectrometry) revealed 128, 121 and 43 proteins differentially expressed in CCA *vs.* control, PSC *vs.* control, and CCA *vs.* PSC groups, respectively. These proteins showed high diagnostic values [maximum of 92.3% sensitivity (SEN), 90.0% specificity (SPE) and an area under the ROC curves (AUC) of 0.983 for CCA *vs.* control, 100% SEN, 90.0% SPE and AUC of 0.967 for PSC *vs.* control, and 83.3% SEN, 88.9% SPE and AUC of 0.898 for CCA *vs.* PSC]. The proteomic analysis of EV isolated from CCA human cells *in vitro* revealed higher abundance of oncogenic proteins compared to EV released by NHC. Orthotopic implant of CCA human cells in the liver of immunodeficient mice resulted in the release of EV containing some similar human oncogenic proteins into the serum.

**Conclusions:** Novel proteomic signatures found in serum EV of CCA and PSC patients show potential usefulness as diagnostic and prognostic tools. CCA-derived EV contain increased concentration of oncogenic proteins that might participate in tumor progression.

**Disclosure of Interest:** None Declared



## The role of ageing in the pathophysiology of the biliary epithelium: identification of a molecular profile

Luca Maroni<sup>1\*</sup>, Claudio Pinto<sup>1</sup>, Debora Maria Giordano<sup>1</sup>, Stefania Saccomanno<sup>1</sup>, Chiara Rychlicki<sup>1</sup>, Luciano Trozzi<sup>1</sup>, Maria Cristina Albertini<sup>2</sup>, Fiorenza Orlando<sup>3</sup>, Espen Melum<sup>4</sup>, Jesus Banales<sup>5</sup>, Antonio Benedetti<sup>1</sup>, Gianluca Svegliati Baroni<sup>1</sup>, Marco Marzioni<sup>1</sup>

<sup>1</sup>Department of Gastroenterology and Hepatology, Università politecnica delle Marche, Ospedali Riuniti university hospital, Ancona, <sup>2</sup>Dipartimento Scienze Biomolecolari, Università degli Studi di Urbino “Carlo Bo”, Urbino, <sup>3</sup>Servizio di Allevamento Sperimentazione Animale, Polo Scientifico Tecnologico, Falconara, Italy, <sup>4</sup>Norwegian PSC Research Center, and the Division of Surgery, Inflammatory Medicine and Transplantation, Oslo University Hospital, Rikshospitalet, Oslo, Norway, <sup>5</sup>Investigator Miguel Servet, Ikerbasque, CIBERehd, Biodonostia Research Institute - Donostia University Hospital, San Sebastian, Spain

**Corresponding author's email:** luca.maroni@live.it

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**Introduction:** Ageing is a complex biological process that affects the functional capacity of multiple organs. Disorders of the biliary tree, such as primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) or cholangiocarcinoma (CCA), develop and progress differently according to the patient age. It is currently not known whether the ageing process affects the response to injury of cholangiocytes.

**Aims:** The aim of the study was to identify molecular pathways associated to cholangiocyte ageing and to verify their effects in the biological response to injury of biliary cells.

**Material and Methods:** A panel of microRNAs (miRs) involved in ageing processes was evaluated in cholangiocytes isolated from 2-month old (young) and 22-month old (old) mice, subjected or not to 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-treatment, a model of sclerosing cholangitis. Intracellular pathways common to elevated microRNAs were identified by *in silico* analysis. Cell proliferation, senescence and senescence-associated secretory phenotype (SASP) were evaluated in Twf1 knocked-down cells. *In vivo*, senescence-accelerated prone mice (SAMP8, a model for accelerated ageing) or controls were subjected to DDC.

**Results:** Cholangiocytes of DDC-treated mice showed up-regulation of ageing-related miR-1a, miR-30e, miR-93, miR-34a, miR-146b and miR-20a. Twinfilin-1 (Twf1) was identified by *in silico* analysis as a common target of the up-regulated miR-1a, miR-30e and miR-20a. Young mice subjected to DDC and old-untreated mice showed similar expression of *Twf1* and related miRs in cholangiocytes, which was markedly increased

as compared to young untreated ones. *Twf1* and miRs expression was further increased in DDC-treated old mice. By immunofluorescence, intrahepatic cholangiocytes of PSC patients stained positive for *Twf1* expression. Knock down of *Twf1* by siRNAs in cultured rat cholangiocytes significantly reduced cell proliferation. In parallel, senescence and SASP markers resulted increased in *Twf1* knocked-down cholangiocytes upon pro-proliferative stimulation compared as to control. In vivo, SAMP8 mice with accelerated ageing showed increased biliary proliferation and fibrosis upon DDC administration compared to control animals.

**Conclusions:** We identified *Twf1* as an important mediator of both cholangiocyte adaptation to ageing processes and response to injury. Our data suggest that disease and ageing might share common pathways, which may unveil novel markers of disease progression or pathways for therapeutic intervention.

**Disclosure of Interest:** None Declared

## Inhibition of Src tyrosine kinase restores CFTR function in cystic fibrosis cholangiocytes derived from human induced pluripotent stem cells (iPSC) and improves the response to CFTR potentiators and correctors used in therapy

Romina Fiorotto\*<sup>1,2</sup>, Mariangela Amenduni<sup>1</sup>, Valeria Mariotti<sup>3</sup>, Luca Fabris<sup>2,3</sup>, Carlo Spirli<sup>1,2</sup>, Mario Strazzabosco<sup>1,2</sup>

<sup>1</sup>Department of Internal Medicine, Section of Digestive Diseases, Yale University, New Haven, United States, <sup>2</sup>International Center for Digestive Health, Department of Medicine and Surgery, University of Milan-Bicocca, Milan, <sup>3</sup>Department of Molecular Medicine, University of Padua, Padua, Italy

Corresponding author's email: [mario.strazzabosco@yale.edu](mailto:mario.strazzabosco@yale.edu)

**Introduction:** Cystic fibrosis associated liver disease (CFLD) is a genetic cholangiopathy that can progress into sclerosing cholangitis or focal biliary cirrhosis. A percentage of CF liver patients present the CFTR mutation  $\Delta F508$  that prevents the trafficking of CFTR to the plasma membrane and causes a gating defect. FDA has recently approved small molecules that correct the F508 deletion (VX809) and potentiate the rescued channel activity (VX770) but their efficacy is lowered by the inflammatory status of the patients and instability of the rescued CFTR at the membrane. Defective secretory function and altered control of epithelial inflammation has been shown to contribute to CFLD, however, the lack of human-derived experimental models has hampered the understanding of CFLD and the search for a cure.

**Aims:** Aim of this study was to use human induced pluripotent stem cells (iPSC) technology to generate a human cell model of CFLD and find targetable pathways for therapy of CFLD.

**Material and Methods:** We devised a protocol to differentiate cholangiocytes from iPSC from normal and homozygous  $\Delta F508$  CFTR individuals.

**Results:** Human iPSC-derived cholangiocytes reproduced the polarity and the secretory function of the biliary epithelium and could be expanded in culture for several passages. As expected,  $\Delta F508$  cholangiocytes showed reduced cAMP-stimulated apical fluid secretion. Administration of VX-770 and VX-809 only slightly improved the fluid secretion.  $\Delta F508$  cholangiocytes showed increased activation of NF- $\kappa$ B in response to LPS, increased phosphorylation of Src kinase and TLR4 and structural alterations of

the F-actin cytoskeleton suggesting that altered control of cholangiocyte innate immune pathways, previously described in mice, is relevant for the human disease. Inhibition of Src significantly decreased NF- $\kappa$ B activation and corrected the cytoskeletal defect. Moreover, when administered along with VX770 and VX809, inhibition of Src restored the normal cholangiocyte secretory function.

**Conclusions:** This study demonstrates that CF biliary cells present changes in biliary innate immunity that reduce the ability to respond to current treatments. Src inhibition normalizes inflammatory and cytoskeletal changes and improves the effects of current CFTR correctors likely by increasing the stability of  $\Delta$ F508 CFTR at the membrane. Our results have strong translational potential and demonstrate the promise of iPSC technology in modeling cholangiopathies.

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**Disclosure of Interest:** None Declared

## Chronic treatment with clinic dose of metformin completely reverses the epithelial to mesenchymal transition (EMT) in human intrahepatic cholangiocarcinomas (iCCAs) by activated AMPK / Foxo3a pathway

Sabina Di Matteo<sup>\*</sup> <sup>1</sup>, Daniele Costantini<sup>1</sup>, Lorenzo Nevi<sup>1</sup>, Federico Giulitti<sup>1</sup>, Jessica Faccioli<sup>1</sup>, Chiara Napoletano<sup>2</sup>, Samira Safarikia<sup>1</sup>, Emy Manzi<sup>3</sup>, Agostino M. Derose<sup>4</sup>, Fabio Meandro<sup>5</sup>, Maria C. Bragazzi<sup>1</sup>, Gianluca Grazi<sup>3</sup>, Pasquale B. Berloco<sup>5</sup>, Felice giuliant<sup>4</sup>, Guido Carpino<sup>6</sup>, Vincenzo Cardinale<sup>1</sup>, Eugenio Gaudio<sup>6</sup>, Domenico Alvaro<sup>1</sup>

<sup>1</sup>Medico-surgical sciences and Biotechnologies, <sup>2</sup>Department of Experimental Medicine, Sapienza University of Rome, <sup>3</sup>Gastroenterology Unit, Regina Elena National Cancer Institute, <sup>4</sup>Surgery, Hepatobiliary Unit, Catholic University of the Sacred Heart School of Medicine, <sup>5</sup>Department of General Surgery and Organ Transplantation, <sup>6</sup>Department of Health Science, Sapienza university of Rome, Rome, Italy

**Corresponding author's email:** sabina.dimatteo@uniroma1.it

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**Introduction:** CCAs are very aggressive cancers with marked resistance to chemotherapeutics. We have previously demonstrated that CCAs are enriched of cancer stem cells expressing EMT traits. We have established primary cell cultures from human intrahepatic CCA (iCCA) subtypes (i.e., mucin and mixed). Treatment with the anti-diabetic drug metformin at approximately 10  $\mu$ M serum concentration has been associated with reduced cancer incidence.

**Aims:** We aimed to evaluate the effects of metformin on proliferation, apoptosis, cell migration and the expression of EMT traits in primary cultures of iCCAs.

**Material and Methods:** Primary iCCA cell cultures (mucin and mixed) were treated with increasing metformin concentrations, from 5 to 1000  $\mu$ M, for 1-4 days, and, successively, with 10  $\mu$ M for a longer period of time up to 2 months to simulate the chronic administration. We evaluated: (i) proliferation by MTS assay; (ii) apoptosis by flow cytometry analysis of Annexin-VFITC/Propidium Iodide (PI); (iii) cell migration by wound-healing assay and invasion assay using matrigel. The expression of Vimentin, E-Cadherin (CDH1), SNAIL1/2, TWIST1, Cytokeratin19, FOXO3a and AMPK genes were analyzed by RT-qPCR, whereas FOXO3a, Cytokeratin19 and Vimentin were analyzed by Immunofluorescence (IF) and Western Blot (WB) assay.

**Results:** Metformin inhibited cell proliferation (MTS assay, population doubling and population doubling time) and induced apoptosis in primary cultures of mucin- and mixed-iCCA; the effects were dose- and time-dependent ( $p < 0.05$  vs. controls). The migration of iCCA cells was also significantly reduced by treatment with metformin at different concentrations, from 5 to 1000  $\mu\text{M}$ . Although the effects of metformin started at 2 days of treatment, after 12 days of treatment, we observed a markedly decrease of mesenchymal and EMT genes ( $p < 0.05$ ), and an increase of both KTR19 and CDH1 genes ( $p < 0.05$ ) in iCCA cells. Metformin also increased the AMPK and Foxo3a mRNA levels ( $p < 0.05$ ). These data were confirmed on a protein level by WB or IF. Furthermore, after 2 months of treatment, especially in mucin-iCCA, KTR19-positive cells constituted the majority of cell cultures by IF ( $p < 0.05$ ), and parallelly Vimentin protein expression decreased respect of untreated cells by WB ( $p < 0.001$ ).

**Conclusions:** We demonstrated that metformin reverses EMT process by activating AMPK- FOXO3A related pathways in primary cultures of human iCCAs. Therefore, metformin could play anticancer effects against human iCCAs with relevant therapeutic implications.

**Disclosure of Interest:** None Declared

# The bile acid receptor TGR5 regulates paracellular permeability and protects the liver through an impact on the tight junction protein JAM-A

Grégory Merlen<sup>1, 2</sup>, José Ursic-Bedoya<sup>1, 2</sup>, Nicolas Kahale<sup>1, 2</sup>, Hayet Simerabet<sup>1, 2</sup>, Isabelle Doignon<sup>1, 2</sup>, Zahra Tanfin<sup>1, 2</sup>, Noémie Pean<sup>1, 2</sup>, Julien Gautherot<sup>1, 2</sup>, Christoph Ullmer<sup>3</sup>, Lydie Humbert<sup>4, 5</sup>, Dominique Rainteau<sup>4, 5</sup>, Doris Cassio<sup>1, 2</sup>, Thierry Tordjmann<sup>1, 2</sup>

<sup>1</sup>Université Paris Sud, <sup>2</sup>INSERM U1174, Orsay, France, <sup>3</sup>Innovation Center, Roche, Basel, Switzerland, <sup>4</sup>Université Pierre et Marie Curie, <sup>5</sup>INSERM U1057, Paris, France

**Corresponding author's email:** gregory.merlen@gmail.com

**Introduction:** TGR5, the bile acid (BA) G-protein-coupled receptor protects the liver against BA overload, although through unknown mechanisms.

**Aims:** Preliminary data suggested that TGR5, highly expressed in biliary epithelial cells, may regulate biliary epithelium permeability.

**Material and Methods:** Trans-epithelial resistance (TER) and 10 kDa fluorescent dextran transfer were measured in the NRC (Normal Rat Cholangiocyte) cell line. To study biliary epithelial permeability in vivo, fluorescent dextran or modified glycocholic acid were injected in the gallbladder (GB) lumen and traced (spectrofluorimetry and Mass Spectrometry) in plasma and liver. Cells and mice were treated with RO5527239, a specific TGR5 agonist, and with taurochenodeoxycholic acid (TLCA). TGR5-induced signaling pathways were studied by western blot (WB). Tight junction (TJ) proteins expression was investigated by qPCR, WB and immunofluorescence in NRC, in livers and GB from WT (Wild Type) and TGR5-KO mice, under vehicle or TGR5 agonist treatment, in basal conditions or after Bile Duct Ligation (BDL).

**Results:** In NRC, TGR5 agonists increased TER, reduced dextran passage, induced ERK phosphorylation and EGFR transactivation. Inhibition of EGFR transactivation suppressed TGR5 agonists' effect on NRC paracellular permeability. BA and dextran transepithelial transfer after GB injection was increased in TGR5-KO as compared with WT mice. In TGR5-KO mice, among the TJ proteins studied, only junctional adhesion molecule A (JAM-A) expression and localization at TJ were reduced in bile ducts and GB epithelia, as compared with WT. In vitro, TGR5 agonists induced JAM-A expression and phosphorylation in NRC cells. In TGR5-transfected cells, increasing JAM-A expression correlated with increasing TGR5 expression and TER. In vivo, JAM-A phosphorylation was induced in GB epithelium after GB lumen TGR5 agonist injection, in WT but not

TGR5-KO mice. After TGR5 agonist treatment, JAM-A expression and phosphorylation increased (biliary tract) and dextran transepithelial transfer decreased (GB injection) in WT but not TGR5-KO mice. After BDL (24-48 h), JAM-A expression and phosphorylation was increased in bile ducts and GB epithelia as compared with control animals, and TGR5 agonist-pre-treated mice were protected from BDL-induced liver injury.

**Conclusions:** The BA receptor TGR5 regulates biliary epithelial permeability in vitro and in vivo, through mechanisms modulating the TJ protein JAM-A expression and phosphorylation, thereby protecting liver parenchyma against bile leakage.

**Disclosure of Interest:** None Declared



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## Combined targeting of cholangiocytes and activated stromal fibroblasts with a Bcl-xL inhibitor ameliorates liver fibrosis in Mdr2<sup>-/-</sup> mice

Anja Moncsek<sup>1</sup>, Mohammed S. Al Surahi<sup>2</sup>, Christy E. Trussoni<sup>2</sup>, Steven P. O'Hara<sup>2</sup>, Patrick L. Splinter<sup>2</sup>, Camille Zuber<sup>1</sup>, Eleonora Patsenker<sup>1</sup>, Piero V. Valli<sup>1</sup>, Christian D. Fingas<sup>3</sup>, Achim Weber<sup>4</sup>, Yi Zhu<sup>5</sup>, Tamar Tchkonja<sup>5</sup>, James L. Kirkland<sup>5</sup>, Beat Müllhaupt<sup>1</sup>, Nicholas F. LaRusso<sup>2</sup>, Joachim C. Mertens<sup>1</sup>

<sup>1</sup>Gastroenterology and Hepatology, University Hospital Zürich, Zürich, Switzerland, <sup>2</sup>Gastroenterology and Hepatology, Mayo Clinic, Rochester, United States, <sup>3</sup>General, Visceral, and Transplantation Surgery, University Hospital Essen, Essen, Germany, <sup>4</sup>Pathology and Molecular Pathology, University Hospital Zürich, Zürich, Switzerland, <sup>5</sup>Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, United States

**Corresponding author's email:** anja.moncsek@usz.ch

**Introduction:** Primary sclerosing cholangitis (PSC) is a chronic cholestatic disorder that is characterized by persistent biliary inflammation and fibrosis. PSC cholangiocytes exhibit a senescence-associated secretory phenotype. The persistent secretion of growth factors such as platelet-derived growth factor (PDGF) by senescent cholangiocytes leads to the activation of fibroblasts. Activated stromal fibroblasts (ASF) are the drivers of fibrosis development. We have previously shown that the survival of ASF is Bcl-xL-dependent *in vitro*. Moreover, senescent cells maintain their viability by upregulation of anti-apoptotic Bcl-xL. Therefore, we hypothesized that inhibition of Bcl-xL leads to depletion of ASF and reduction of growth factor-induced fibroblast activation by senescent cholangiocytes and finally to a reduction of biliary fibrosis.

**Material and Methods:** PDGF-activated fibroblasts and senescent-induced cholangiocytes were treated with a BH3-mimetic specific for Bcl-xL (A-1331852) or siRNA. Apoptosis induction was analyzed by caspase-3/7 activity assay and DAPI staining. Mdr2<sup>-/-</sup> mice were treated with A-1331852 for 14 days by daily oral gavage. Subsequently, liver fibrosis was quantified by hydroxyproline assay and Sirius red staining. Cholangiocyte senescence was analyzed by p16 FISH. Gene expression of fibrosis-associated growth factors and  $\alpha$ SMA was investigated by qPCR.

**Results:** We identified Bcl-xL as a key survival factor in PDGF-activated fibroblasts. Bcl-xL was also upregulated in senescent cholangiocytes. Inhibition of Bcl-xL by A-1331852

or siRNA significantly increased caspase-3/7 activity as well as the number of apoptotic nuclei in PDGF-activated fibroblasts compared to quiescent counterparts. Additionally, inhibition of Bcl-xL specifically reduced the survival of senescent cholangiocytes. Treatment of MDR2<sup>-/-</sup> mice with A-1331852 resulted in a significant reduction of liver fibrosis as shown by Sirius red staining and hydroxyproline quantification. This was accompanied by a reduction in the number of senescent cholangiocytes and a significantly reduced expression of fibrosis-inducing growth factors as well as  $\alpha$ SMA in A-1331852-treated Mdr2<sup>-/-</sup> mice compared to vehicle treated mice.

**Conclusions:** Treatment of liver fibrosis with Bcl-xL inhibitor A-1331852 leads to a reduction of fibrosis *in vivo*, possibly due to a) depletion of ASF and b) reduction of senescent cholangiocytes. This proposed dual mechanism makes specific Bcl-xL inhibition an attractive therapeutic strategy in biliary fibrosis.

**Disclosure of Interest:** None Declared

## ATP Binding Cassette B4 (ABCB4) related disease – from genetics to phenotypic variability: a case series

Pedro Marques da Costa\*<sup>1</sup>, Carlos Noronha Ferreira<sup>1</sup>, Helena Cortez-Pinto<sup>1</sup>, José Velosa<sup>1</sup>

<sup>1</sup>Gastroenterology and Hepatology, Hospital Santa Maria, Lisboa, Portugal

Corresponding author's email: pedroavmcosta@gmail.com

**Introduction:** The ABCB4 gene encodes the multidrug resistance protein 3 (MDR3), a canalicular membrane flippase involved in bile formation. ABCB4 mutations have been associated with progressive familial intrahepatic cholestasis type 3 (PFIC3); intrahepatic cholestasis of pregnancy (ICP); estrogen-induced cholestasis (EIC) and low phospholipid associated cholelithiasis (LPAC).

**Aims:** Describing genotypic and phenotypic associations in patients with ABCB4 mutations.

**Material and Methods:** We reviewed the medical records; laboratory analysis, radiological and endoscopic examinations as well as RT-PCR gene sequencing analysis from patients diagnosed with ABCB4 mutations, at our center, within the past 5 years.

**Results:** Five patients were included. Median time to diagnosis: 8.8 years (IQR:8.76-24.6). *Patient 1:* 27-year-old female submitted to cholecystectomy (COT) at age 16 due to cholelithiasis. At age 18 had an EIC episode followed by two episodes of ICP (age 20 and 27) the last requiring plasmapheresis. Between episodes she had pruritus and fluctuating aminotransferase and  $\gamma$ GT elevations. Liver biopsy: portal and peri-portal fibrosis. Genetics: compound heterozygosity with c.959C>T (missense mutation) and c.2682+1G>A (splice site). *Patients 2 and 3:* 55 and 29-year-old females, mother and daughter, respectively. Both were COT before age 25 due to cholelithiasis. Patient 2 further endured 3 ERCs for recurrent cholelithiasis. Patient 3 developed ICP. Both had persistent aminotransferase and  $\gamma$ GT elevation. Genetics: stop codon mutation c475C>T in heterozygosity with a single nucleotide polymorphism (SNP) c.504C>T. *Patient 4:* 36 years-old female had a 10-years history of pruritus and fluctuating aminotransferase and  $\gamma$ GT elevations. No history of cholelithiasis, but an ICP diagnosis on a second pregnancy. Liver biopsy: no relevant findings. Genetics: compound heterozygosity c.711A>T (SNP) and c.1954A>G (conditional SNP). *Patient 5:* 78 years-old female COT at age 54.

Subsequently, she underwent 12 ERCPs for recurrent cholelithiasis, exhibiting persistent  $\gamma$ GT elevation between episodes. Genetics: c.504C>T (SNP associated with increased cholelithiasis).

Patients 1 to 4 showed improvement of liver enzymes after initiating ursodesoxicolic acid (UDCA).

**Conclusions:** ABCB4 related disease demonstrates a high genotypic and phenotypic variability contributing to a significant delay in diagnosis. Recurrent cholelithiasis seems to be a prevalent phenotypic trait. Haplotype (more than type of mutation) relates with more severe disease expressions.

**Disclosure of Interest:** None Declared

Figure:

Patient	Sex	Age at first presentation	Family history	Alkaline phosphatase elevation	gGT elevation	Pruritus	Cholestasis	CDT	ICP	EC	Time to diagnosis (months)	Haplotype	Mutation	Type of mutation
1	F	16	N	S	S	S	S	S	S	S	106.0	CH	c.395C>T c.2582T>G>A	(missense) (splice site)
2	F	19	S	S	S	N	S	S	N	N	450.1	H	c.475C>T	(stop codon)
3	F	27	S	S	S	S	S	S	S	N	13.9	CH	c.475C>T c.504C>T	(stop codon) (SNP)
4	F	26	N	S	S	S	N	N	S	N	105.1	CH	c.711A>T	(SNP)
5	F	34	N	N	S	N	S	S	N	N	293.2	H	c.1195A>G c.304C>T	(conditional SNP) (SNP)
<b>Total</b>	<b>NA</b>	<b>24 ± 3.5</b>	<b>40%</b>	<b>80%</b>	<b>100%</b>	<b>60%</b>	<b>80%</b>	<b>80%</b>	<b>60%</b>	<b>20%</b>	<b>106.0 (Q1-13.8)</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>

Figure 3. Clinical and biochemical characteristics of the patients. CDT, cholelithiasis; ICP, intrahepatic cholestasis of pregnancy; EC, extrahepatic cholestasis; CH - compound heterozygosity; H - simple heterozygosity. Values are for nominal variables (%), for continuous variables: mean ± SD. For age and median and IQR for time to diagnosis. NA, not applicable.

## Autotaxin activity predicts transplant-free survival in primary sclerosing cholangitis

Johannes R. R. Hov\*<sup>1, 2</sup>, Andreas E. Kremer<sup>3</sup>, Kirsten M. Boberg<sup>1, 2, 4</sup>, Ronald P. Oude Elferink<sup>5</sup>, Tom H. Karlsen<sup>1,2,4</sup>, Ulrich Beuers<sup>5</sup>, Mette Vesterhus<sup>1,6</sup>

<sup>1</sup>Norwegian PSC Research Center, Dept of transplantation medicine, Oslo University Hospital Rikshospitalet, <sup>2</sup>Institute of Clinical Medicine, University of Oslo, Oslo, Norway, <sup>3</sup>Department of Medicine 1,, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany, <sup>4</sup>Section of Gastroenterology, Department of Transplantation Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway, <sup>5</sup>Department of Gastroenterology and Hepatology and Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, <sup>6</sup>National Centre for Ultrasound in Gastroenterology, Haukeland University Hospital, Bergen, Norway

**Corresponding author's email:** [j.e.r.hov@medisin.uio.no](mailto:j.e.r.hov@medisin.uio.no)

**Introduction:** Autotaxin is a secreted enzyme responsible for generating extracellular lysophosphatidic acid, and has been linked to cholestatic pruritus. Recently, serum autotaxin activity was described as a marker of severity of liver injury and overall survival in patients with primary biliary cholangitis and primary sclerosing cholangitis (PSC).

**Aims:** In the present study we aimed to validate these observations in two independent Norwegian PSC panels and explore the role of this new biomarker.

**Material and Methods:** In panel 1, collected between 1992-2006, 165 (74% male) patients with large duct PSC were included, while panel 2 comprised 87 (81% male) patients collected from 2008-2011. Stored sera thawed up to two times were analyzed for autotaxin activity by a fluorometrix enzymatic assay. Liver transplant-free survival was analyzed using Kaplan-Meier plots and univariate and multivariate Cox regression after a median observation time of 3.2 years (range 0.0-20 years).

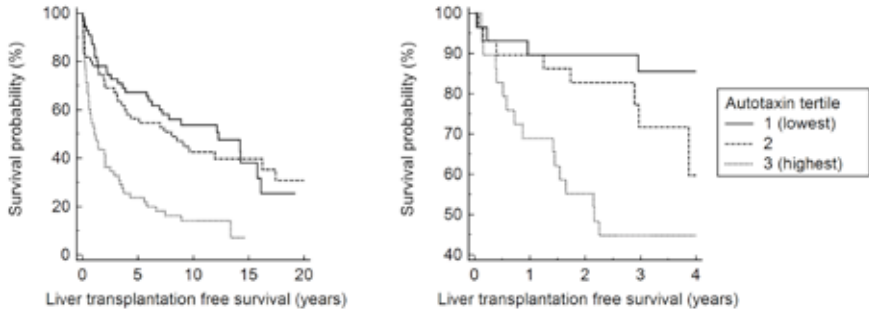
**Results:** When categorizing the patients according to tertiles of autotaxin activity, there was a strong association between increasing autotaxin level and shorter liver transplantation free survival in both panel 1 and panel 2, as shown in the left and right parts of the figure, respectively ( $p < 0.001$  for both). Mayo risk scores were available in 81% of these patients, and were moderately correlated to autotaxin (Spearman's rho 0.41,  $p < 0.001$ ). The panels were merged to increase power when exploring the relationship to other biomarkers, and analyzed using Cox regression. Both Mayo risk score and autotaxin activity (log-



transformed) were independently associated with an increased risk of liver transplantation or death (OR 1.72 (95% CI 1.52-1.95),  $p < 0.001$  and OR 1.91 (95% CI 1.18-3.09),  $p = 0.009$ , respectively).

**Conclusions:** Increased serum autotaxin activity is associated with reduced liver transplant-free survival in PSC independent from Mayo risk score.

**Figure:**



**Disclosure of Interest:** None Declared

## Natural Killer T cells are activated in mice with cholestasis

Laura Valestrand\*<sup>1,2,3</sup>, Natalie L. Berntsen<sup>1,2,3</sup>, Bjarthe Fosby<sup>4</sup>, Tom H. Karlsen<sup>1,2,3,5</sup>, Pål D. Line<sup>3,4</sup>, Xiaojun Jiang<sup>1,2</sup>, Espen Melum<sup>1,2,5</sup>

<sup>1</sup>Norwegian PSC Research Center, <sup>2</sup>Research Institute of Internal Medicine, Division of Surgery, Inflammatory Medicine and Transplantation, Oslo University Hospital, Rikshospitalet, <sup>3</sup>Institute of Clinical Medicine, University of Oslo, <sup>4</sup>Section of Transplantation Surgery, <sup>5</sup>Section of Gastroenterology, Division of Surgery, Inflammatory Medicine and Transplantation, Oslo University Hospital, Rikshospitalet, OSLO, Norway

**Corresponding author's email:** lauravalestrand@gmail.com

**Introduction:** Natural Killer T (NKT) cells are a subset of lymphocytes with regulatory properties. They respond to lipid antigens presented by the non-classical MHC molecule CD1d. We have previously demonstrated that CD1d on cholangiocytes present lipid antigens to NKT cells.

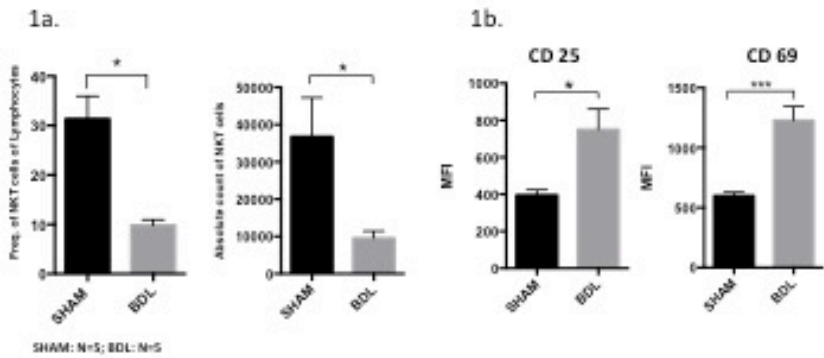
**Aims:** In this project we investigate the role of NKT cells in the pathophysiology of cholestasis by ligating the common bile duct of mice.

**Material and Methods:** We ligated the common bile duct of mice and harvested livers and spleens after 3 and 5 days. Control mice underwent an identical surgical procedure only without bile duct ligation. Isolated lymphocytes from liver and spleen were stained with monoclonal antibodies and analysed by flow cytometry.

**Results:** After bile duct ligation we found decreased hepatic NKT cells, which is a sign of activation, after 5 days (33% vs.15%,  $P=0.02$ ) in mice with cholestasis with a trend already at 3 days (23% vs.16%,  $P=0.31$ ). There was no change in the percentage of TCRb<sup>+</sup> cells that were not NKT-cells. Specifically, the CD4-positive NKT cells were reduced (75% vs. 48%,  $P=0.05$ ). The NKT cells in cholestatic mice had an activated phenotype with increased CD69 expression at both day 3 (median fluorescence intensity (MFI) 709 vs. 1458,  $P < 0.001$ ) and day 5 (599 MFI vs. 1229 MFI,  $P < 0.001$ ). The remaining T-cell population in the liver and in the spleen did not display an activated phenotype.

**Conclusions:** In mice with cholestasis we found a reduced intrahepatic NKT cell population that were activated. This reduction may be perceived as part of the activation process where the T-cell receptor is down regulated. These findings indicate that NKT cells are activated during cholestasis and likely play a role in the pathophysiology of cholestasis.

**Figure:**



**Figure 1.** Liver NKT cell population size and activation 5 days after bile duct ligation. (a.) Reduction of frequency and absolute count of NKT cells in cholestatic mice. (b.) Increased mean fluorescence intensity (MFI) of the activation markers CD 25 and CD 69 in cholestatic mice.

**Disclosure of Interest:** None Declared

## Auto-antibodies of the IgG4 and IgG1 subclass against Annexin A11 in IgG4-related disease of the biliary tract and pancreas

Lowiek Hubers\*<sup>1</sup>, Harmjan Vos<sup>2</sup>, Alex R. Schuurman<sup>1</sup>, Robin Erken<sup>1</sup>, Ronald P. Oude Elferink<sup>1</sup>, Boudewijn M. Burgering<sup>2</sup>, Stan F. Van de Graaf<sup>1</sup>, Ulrich Beuers<sup>1</sup>

<sup>1</sup>Gastroenterology and Hepatology/Tytgat Institute, Academic Medical Centre, Amsterdam,

<sup>2</sup>Center for Molecular Medicine, University Medical Centre, Utrecht, The Netherlands


Corresponding author's email: l.m.hubers@amc.uva.nl

**Introduction:** Immunoglobulin G4-related disease is an increasingly recognized immune-mediated disorder involving various organs, including the pancreas (autoimmune pancreatitis) and biliary tract (IgG4-associated cholangitis). Typically, IgG4 serum levels are elevated and infiltrating IgG4-producing B and plasma cells can be found in affected tissues. IgG4 antibodies possess certain biological properties that are considered anti-inflammatory, but the role that IgG4 plays in IgG4-related disease remains enigmatic. Recently, we identified a limited number of highly expanded IgG4+ B cell receptor clones in blood and affected tissue of patients with active IgG4-related disease of the pancreas and biliary tract, suggesting that specific antigenic stimuli are involved.

**Aims:** This research project aimed to identify possible target (auto)antigens for IgG4 that may be involved in the immune response in IgG4-related disease.

**Material and Methods:** We screened sera of patients with IgG4-associated cholangitis and/or autoimmune pancreatitis (n=50), for reactivity against a H69 cholangiocyte cell line lysate on immunoblot. Sera of patients with primary sclerosing cholangitis (n=20) or pancreatobiliary malignancies (n=27) were used as controls. Subsequently, IgG4-antigens were immunoprecipitated using IgG4-capturing beads and identified by quantitative mass-spectrometry.

**Results:** We found that IgG4, but also IgG1, from sera of patients with IgG4-related disease of the biliary tract and pancreas (n=7), but not of controls, showed reactivity against a  $\pm$  56 kDa cytosolic protein. Peptide mass fingerprinting identified Annexin A11, belonging to the Annexin family of calcium-dependent phospholipid binding proteins. The specificity of the patient sera for Annexin A11 was confirmed by screening reactive sera against an Annexin A11 knockdown cell lysate on immunoblot and against recombinant



Annexin A11 in an enzyme-linked immunosorbent assay. Furthermore, IgG4 antibodies blocked binding of IgG1 antibodies to Annexin A11 in a competition ELISA.

**Conclusions:** Autoantibodies against Annexin A11 may be involved in initiating and/or maintaining the immune response in IgG4-related disease of the biliary tract and pancreas. Annexin A11-specific IgG4 may be upregulated to dampen an IgG1-mediated pro-inflammatory immune response against Annexin A11, in line with a presumed anti-inflammatory action of IgG4 in IgG4-related disease.

**Disclosure of Interest:** None Declared

## Circulating cancer-associated large extracellular vesicles in cholangiocarcinoma

Sabine K. Urban<sup>1</sup>, Henrike Julich-Haertel<sup>1</sup>, Marcin Krawczyk<sup>1,2</sup>, Arnulf Willms<sup>3</sup>, Krzysztof Jankowski<sup>4</sup>, Waldemar Patkowski<sup>5</sup>, Robert Schwab<sup>3</sup>, Ines Richardsen<sup>3</sup>, Sebastian Schaaf<sup>6</sup>, Sebastian Gehlert<sup>6</sup>, Jesus M. Banales<sup>7</sup>, Piotr Milkiewicz<sup>8,9</sup>, Frank Lammert<sup>1</sup>, Marek Krawczyk<sup>5</sup>, Veronika Lukacs-Kornek<sup>1</sup>, Miroslaw Kornek<sup>1,3</sup>

<sup>1</sup>Department of Medicine II, Saarland University Medical Center, Homburg, Germany,

<sup>2</sup>Laboratory of Metabolic Liver Diseases, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Warsaw, Poland, <sup>3</sup>Department of General, Visceral and Thoracic Surgery, German Armed Forces Central Hospital, Koblenz, Germany, <sup>4</sup>Department Internal Medicine and Cardiology, <sup>5</sup>Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Warsaw, Poland, <sup>6</sup>Department of Molecular and Cellular Sport Medicine, Institute of Cardiovascular Research and Sport Medicine, German Sport University Cologne, Cologne, Germany, <sup>7</sup>Department of Liver and Gastrointestinal Diseases, Biodonostia Health Research Institute – Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastian, Spain, <sup>8</sup>Liver and Internal Medicine Unit, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Warsaw, <sup>9</sup>Translational Medicine Group, Pomeranian Medical University, Szczecin, Poland

**Corresponding author's email:** urban.sabine@gmx.net

**Introduction:** Considering high fatalities and a lack of current early detection options, liver cancer presents as a severe disease with high mortality rates. A new approach for detection of cancers involves tumor-associated microparticles (taMPs), large extracellular vesicles, that feature the same surface antigen composition as their parental cells and circulate in the bloodstream.

**Aims:** Here, we aim to improve early detection and therapy monitoring possibilities of cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC) by applying this novel, minimally-invasive approach.

**Material and Methods:** TaMPs from patients' sera were isolated by ultracentrifugation. FACS methodology was applied to identify taMP populations that were associated with the presence of liver tumors or with cirrhosis, a non-tumor related disease. In total, 172 patients with liver cancer (CCA or HCC), 54 patients with cirrhosis and 202 control

subjects were enrolled in the study. In 27 liver cancer patients a R0 resection was performed and taMP levels were determined until 10 days after surgery.

**Results:** Adding to previous results, the list of detectable cancers by AnnexinV+EpCAM+CD147+ taMPs was extended by CCA and HCC. Moreover, AnnexinV+EpCAM+CD133+ and AnnexinV+EpCAM+ASGPR1+CD133+ taMPs allowed the distinction of liver disorders (including CCA, HCC and cirrhosis) from tumor-free individuals and from patients carrying non-liver cancers. Additionally, AnnexinV+EpCAM + ASGPR1 + taMPs were increased in liver cancer-bearing patients by 3.05-fold ( $p < 0.0005$ ) as compared to cirrhosis patients that lacked any tumor. AUROC value, sensitivity (75%) and specificity as well as positive (78%) and negative predictive value for liver tumors (CCA and HCC combined) indicated a potent diagnostic accuracy of this taMP population. Also, AnnexinV+ EpCAM+ ASGPR1 + taMPs decreased from 26.7 (pre-R0 resection) to 16.1 (day 2 post-R0 resection,  $p < 0.005$ ) and remained low at 7.7 (day 10 post-R0 resection,  $p > 0.05$ ) taMPs per  $10^3$  AnnexinV+ MPs. The smallest detectable liver tumors were 9 mm (HCC) and 11 mm (CCA) in size.

**Conclusions:** Our results provide strong evidence that AnnexinV+ EpCAM + ASGPR1 + taMPs are a novel biomarker for CCA and HCC detection. Their assessment reveals the presence and possibly the extent of these cancers. Thus, they represent a minimally-invasive, accurate liquid biopsy screening tool that could improve (early) diagnostics and therapy in patients with primary liver cancers. Additionally, taMPs could be suitable for monitoring anti-tumoral therapy responses.

**Disclosure of Interest:** None Declared

## Cellular senescence exacerbates injury and impairs regeneration in biliary disease

Sofia Ferreira-González<sup>1</sup>, Wei-Yu Lu<sup>2</sup>, Alexander Raven<sup>2</sup>, Tak Yung Man<sup>2</sup>, Eoghan O'Duibhir<sup>2</sup>, Benjamin Dwyer<sup>2</sup>, Tim Kendall<sup>3</sup>, Nuria Tarrats<sup>4</sup>, Juan-Carlos Acosta<sup>4</sup>, Thomas Bird<sup>5</sup>, Stuart Forbes<sup>2</sup>

<sup>1</sup>CRM Centre for Regenerative Medicine, Edinburgh, <sup>2</sup>CRM Centre for Regenerative Medicine, <sup>3</sup>MRC Centre for Inflammation Research, <sup>4</sup>The Institute of Genetics and Molecular Medicine, Edinburgh, <sup>5</sup>The Beatson Institute for Cancer Research, Glasgow, United Kingdom

**Corresponding author's email:** s1340464@sms.ed.ac.uk

**Introduction:** Senescence is a highly efficient mechanism that provides an irreversible barrier to cell cycle progression to prevent undesired proliferation. However, under pathological circumstances, senescence can adversely affect organ function, viability and regeneration. In the context of biliary disease, we hypothesize that senescence is initiated in the bile ducts and spreads to the liver parenchyma, impairing the liver's regenerative capacity and aggravating the condition.


### Aims:

1. To generate an in vivo model that displays senescence in cholangiocytes.
2. To study the response of senescent cholangiocytes during biliary injury.
3. To study the regenerative capacity of the parenchyma in the model.
4. To find potential targets that disrupt the senescent response and rescue the model's phenotype.

**Material and Methods:** We have developed a mouse model of biliary senescence, based on the conditional deletion of Mdm2 in bile ducts that mimics the clinical outcome of biliary disease. Using this model, we studied the underlying mechanisms that characterize biliary disease, and established an essential role of TGFβ in paracrine senescence-associated regeneration. Lastly, we disrupted TGFβ signaling to therapeutically rescue this phenotype in our model of biliary senescence.

**Results:** Our results reveal the detrimental role of senescence in biliary disease, and a TGFβ-dependent mechanism for dissemination of senescence from the biliary epithelium to the parenchyma, impairing liver function. Finally, we have identified TGFβ





signalling disruption as a potential therapeutic target to limit senescence-dependent aggravation in human cholangiopathies.

**Conclusions:** We have developed a highly efficient model for the induction of senescence in cholangiocytes, that partially reflects the clinical symptoms of human biliary disease. This model provides an ideal tool for the study of biliary senescence mechanisms in the liver.

Overall, we have shown in our model that in biliary injury, cellular senescence is likely to be a driver of, rather than simply a consequence of, the disease causing increased fibrosis, reduced regeneration and worsening liver function.

**Disclosure of Interest:** None Declared

## CHOP deficiency protects against bile duct ligation-induced cholestatic liver injury by rescuing ER stress-induced loss of intestinal epithelial stemness

Runping Liu<sup>\*1</sup>, Xiaojiaoyang Li<sup>1</sup>, Phillip B. Hylemon<sup>1</sup>, Huiping Zhou<sup>1</sup>

<sup>1</sup>Microbiology and Immunology, Virginia Commonwealth University, Richmond, United States


Corresponding author's email: huiping.zhou@vcuhealth.org

**Introduction:** Activation of endoplasmic reticulum (ER) stress and inflammatory response have been identified to contribute to cholestatic liver injury. The impaired intestinal barrier function is closely associated with cholestatic disease progression and ablation of CHOP (C/EBP homologous protein) reduced cholestatic liver injury. However, the underlying mechanism remains unclear.

**Aims:** The aim of the current study is to investigate the mechanisms underlying CHOP deficiency-mediated protective effects against BDL-induced cholestatic liver injury.

**Material and Methods:** Wild type and CHOP<sup>-/-</sup> mice were subjected to sham operation or BDL for 2-weeks. The cholestatic liver injury and intestinal barrier function were examined by measuring liver functional enzyme activities and intestinal permeability as well as histological analysis. In addition, isolated intestinal crypts and *in vitro* cultured primary intestinal organoids were used to determine the role of CHOP in regulating intestinal stem cell proliferation and differentiation. The mRNA levels of target genes were determined by real-time RT-PCR. The immunofluorescence staining was used to detect the intestinal epithelial structural proteins and hepatic inflammatory cell infiltration.

**Results:** BDL-induced cholestatic liver injury was significantly reduced in CHOP<sup>-/-</sup> mice. BDL-induced CHOP expression in intestinal epithelium contributed to disruption of intestinal barrier function, bacteria translocation, hepatic infiltration of inflammatory cells and activation of profibrotic response by suppressing intestinal stem cell proliferation and differentiation. Bacteria liposaccharide (LPS) and ER stress inducer thapsigargin (TG) directly inhibited intestinal stem cell proliferation and differentiation, which was prevented by CHOP deficiency.



**Conclusions:** ER stress-mediated rapid loss of intestinal epithelial stemness represents an important mechanism underlying BDL-induced disruption of gut-liver axis and cholestatic liver injury.

**Disclosure of Interest:** None Declared

## Role of endoscopic ultrasound with fine needle aspiration for non mass-forming cholangiocarcinomas

Leonardo Henry Eusebi<sup>1, 2</sup>, Andrea Telese<sup>\* 2</sup>, Stephen P. Pereira<sup>2</sup>, Reena Ravikumar<sup>3</sup>, Giuseppe Fusai<sup>3</sup>, Massimo Pinzani<sup>2</sup>, Franco Bazzoli<sup>1</sup>, Douglas Thorburn<sup>2</sup>

<sup>1</sup>Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy, <sup>2</sup>The Royal Free Hospital and University College London, Institute for Liver and Digestive Health, <sup>3</sup>Department of HPB and Liver Transplant Surgery, The Royal Free Hospital and University College London, London, United Kingdom


**Corresponding author's email:** leonardo.eusebi@unibo.it

**Introduction:** Preoperative pathological diagnosis in patients with biliary strictures suspected for malignancy is often difficult. The sensitivity of endoscopic retrograde cholangio-pancreatography (ERCP) guided brushing and biopsy is low. Endoscopic ultrasound fine needle aspiration (EUS-FNA) may be difficult to perform in non-mass forming lesions and data on its role in this setting are limited.

**Aims:** The aim of the present study was to evaluate the efficacy and safety of EUS-FNA for preoperative evaluation and ability to make cytological diagnosis of non mass-forming cholangiocarcinomas (CC).

**Material and Methods:** Subjects that had undergone surgery for CC as well as EUS-FNA of the lesion before surgery and did not have a visible mass on pre-procedure CT scan or MRI imaging and on EUS examination were included. Diagnostic accuracy was defined as the frequency of cases correctly diagnosed by EUS-FNA using surgical pathology as the reference standard. Secondary outcome was evaluation of EUS-FNA safety in this setting, which was defined as the presence of complications within 48 hours following the procedure.

**Results:** From June 2012 to December 2016 at the HPB and Liver Transplant Surgery Department of the Royal Free Hospital, 124 patients had been operated for cholangiocarcinoma, of which 13 had previously undergone EUS-FNA. Eight of these cases (5 males, mean age of 72, range 65 to 78) had a non mass-forming CC and were included in the analysis. In 6 cases, FNA was performed using a 25-gauge needle (3 Procore, Cook Endoscopy, 3 Expect, Boston Scientific) and 2 cases with a 22-gauge



needle (1 Procore, 1 Expect), with median number of 3 passes obtained from the bile duct walls (range 2-4). Cytological diagnosis of FNA specimens was positive for malignancy in 6 patients and suspicious for malignancy in 1 patient. In one patient the cytological result was inconclusive. Considering only the positive cytology cases, the sensitivity of EUS-FNA was 75%, and was higher for distal CCs (83.3%, 5/6) than for proximal CCs (50%, 1/2). Considering also the suspicious cases, the sensitivity reached 87.5%. No complications were reported.

**Conclusions:** EUS-FNA is a safe and accurate procedure in the setting of non mass-forming cholangiocarcinomas. EUS should be performed in patients with suspected malignant biliary obstruction as it can impact on clinical and therapeutic decision-making.

**Disclosure of Interest:** None Declared

## Early identification of candidates for second-line therapies in PBC using a “UDCA-response” model

Marco Carbone\*<sup>1</sup>, Alessandra Nardi<sup>1</sup>, Dave Jones<sup>2</sup>, George Mells<sup>1</sup> and UK-PBC Consortium

<sup>1</sup>Academic Department of Medical Genetics, University of Cambridge, Cambridge, <sup>2</sup>Institute of Cellular Medicine, Newcastle University, Newcastle, United Kingdom

Corresponding author's email: mc767@medschl.cam.ac.uk

**Introduction:** The FDA and EMA have granted accelerated approval for OCA as add-on therapy for PBC patients with inadequate response to treatment with UDCA at an optimal dose for  $\geq 12$  months. In essence, this approach means that patients at highest risk of disease progression – those with active disease and no discernible response to UDCA – are left untreated for an additional year while they prove their need for second-line therapy. Predicting at baseline the likelihood of response to first-line treatment with UDCA would enable clinicians to prioritize patients at highest risk for add-on therapy from the outset.

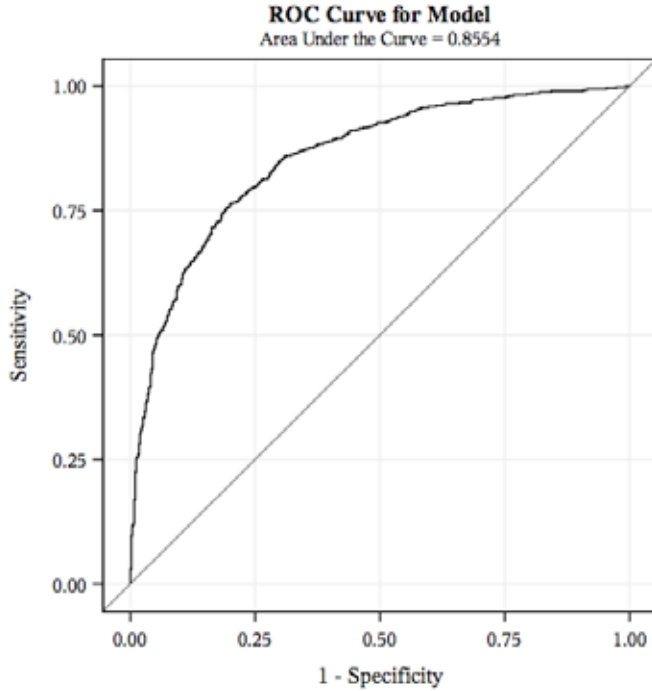
**Aims:** The aim of our study was to identify baseline variables that predict UDCA response and thereby develop a model of treatment response.

**Material and Methods:** We analyzed data on 3062 patients from the UK-PBC Research Cohort treated with ursodeoxycholic acid (UDCA). We performed logistic regression analysis of diverse explanatory variables to derive the best-fitting model. The endpoint was UDCA response, defined as an ALP  $< 1.67$  after at least 12 months of UDCA therapy.

**Results:** The median follow-up was 7.4 years (interquartile range: 4.2, 10.8). The rate of UDCA response was 78%. The most important baseline predictors of UDCA response were as follows: alkaline phosphatase (odds ratio [OR] 0.09, confidence interval [CI] 0.07-0.11);  $\sqrt{1}$ /bilirubin (OR 1.76, CI 1.32-2.36); transaminases (OR 1.4, CI 1.15-1.7), and age (OR 1.03, CI 1.02-1.04). We included these variables in a predictive model (UDCA-response model) that we subjected to internal cross-validation, which predicted future response to UDCA with an area under receiver operating curve (AUROC) of 0.86 (linear predictor:  $0.557 + 0.57(\sqrt{\text{Bil}}) - 2.41 * \log \text{ALP} + 0.33 * \log \text{transaminasis} + 0.03 * \text{age}$ ). For a probability level of 0.65, the sensitivity was 82.5 and specificity, 71.4.

**Conclusions:** We developed and validated a model predicting future response to UDCA. The *UDCA-response* model allows for baseline risk stratification and selection of patients for add-on therapy. Notably, raised transaminases at baseline, not related to autoimmune hepatitis overlap, in non-cirrhotic PBC patients is a marker of potential for response to UDCA treatment and therefore better outcome.

**Figure:**



**Disclosure of Interest:** None Declared

## microRNA-21 is overexpressed in primary biliary cholangitis and contributes to liver injury and necroptosis in cholestatic bile duct-ligated mice

Marta B. Afonso<sup>\*1</sup>, Pedro M. Rodrigues<sup>1</sup>, André L. Simão<sup>1</sup>, Maria M. Gaspar<sup>1</sup>, Jesús M. Bañales<sup>2,3,4</sup>, Rui E. Castro<sup>1</sup>, Cecília M. P. Rodrigues<sup>1</sup>

<sup>1</sup>Research Institute for Medicines (iMed.U LISboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal, <sup>2</sup>Department of Liver and Gastrointestinal Diseases, Biodonostia Research Institute – Donostia University Hospital – University of the Basque Country (UPV/EHU), San Sebastian, <sup>3</sup>National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd, Instituto de Salud Carlos III), Madrid, <sup>4</sup>IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

**Corresponding author's email:** mbafonso@ff.ulisboa.pt


**Introduction:** Inhibition of microRNA-21 (miR-21) prevents necroptosis in the mouse pancreas and protects from fibrosis in multiple organs. In turn, we recently showed that necroptosis and the receptor-interacting protein 3 (RIP3) kinase contribute to hepatic necroinflammation in the common bile duct ligation (BDL) murine model.

**Aims:** We aimed to evaluate the role of miR-21 in mediating deleterious processes associated with cholestatic liver disease.

**Material and Methods:** The functional crosstalk between miR-21 and necroptosis was investigated *in vitro*. miR-21 expression was evaluated in the liver of PBC patients. Female C57BL/6 wild-type (WT) or miR-21-deficient (miR-21<sup>-/-</sup>) mice were subjected to common bile duct ligation (BDL) or sham surgeries, with biochemical, molecular and histological analysis of hepatic damage, fibrosis, necroptosis and bile acid metabolism, after either acute (3 days) or chronic (14 days) injury.

**Results:** Studies in depleted miR-21 and RIP3 primary mouse hepatocytes established a functional link between miR-21 and necroptosis through cyclin dependent kinase 2 associated protein 1 (CDK2AP1). miR-21 expression increased in the liver of PBC patients and BDL WT mice at both 3 and 14 days. Remarkably, BDL miR-21<sup>-/-</sup> mice displayed decreased circulating levels of hepatic enzymes, compared with WT mice. Deletion of miR-21 also impaired hepatocellular degeneration and fibrogenic gene expression at both time-points, without impacting on inflammation. Hallmarks of necroptosis, including





retention of RIP3 and mixed lineage kinase domain-like (MLKL) phosphorylation in insoluble aggregates were decreased in the liver of BDL miR-21<sup>-/-</sup> mice, concomitantly with decreased hepatic heme oxygenase expression, iron accumulation, and oxidative stress. Finally, miR-21<sup>-/-</sup> mice displayed an improved adaptive response in the expression of bile acid homeostasis-associated genes, such as nuclear receptors, and in bile acid detoxification, uptake and secretion genes.

**Conclusions:** In conclusion, miR-21 ablation ameliorates liver damage and necroptosis in BDL mice; as such, inhibition of miR-21 should arise as a promising approach to treat cholestatic liver diseases. Supported by PTDC/BIM-MEC/0895/2014, SFRH/BD/91119/2012, FCT, Portugal.

**Disclosure of Interest:** None Declared

## Serial changes in liver stiffness measured by transient elastography and acoustic radiation force impulse imaging in patients with cholestasis

Mira A. Shafik\*<sup>1</sup>, Iman Ramzy<sup>1</sup>, Naglaa Zayed<sup>1</sup>, Samar K. Darwesh<sup>1</sup>, Sherief Musa<sup>1</sup>, Ayman Yosry<sup>1</sup>

<sup>1</sup>hepatogastroenterology, Kasr Alainy hospital, Cairo, Egypt


Corresponding author's email: mira.atef@hotmail.com

**Introduction:** Transient elastography (TE) and acoustic radiation force impulse (ARFI) imaging are non-invasive tools to measure liver stiffness (LS), which may be influenced by cholestasis.

**Aims:** The aim of our study was to evaluate the performance of TE and ARFI in cholestatic diseases and to correlate serial changes in LS with biochemical activity

**Material and Methods:** Fifty patients with intrahepatic cholestasis and 38 patients with extrahepatic cholestasis were prospectively enrolled and underwent TE and ARFI. Liver biopsy was done for 35 patients to detect the etiology of intrahepatic cholestasis. Serial changes in LS were evaluated after medical treatment or biliary drainage and correlated with biochemical activity. Analyses to determine the optimal ARFI cut-off values were performed according to stages of clinical interest.

**Results:** In extrahepatic cholestasis, biliary drainage led to a reduction of bilirubin by 7.7 to 2.2 mg/dL which was significantly correlated with a reduction of LS by TE from  $12.38 \pm 6.68$  kPa to  $8.08 \pm 3.21$  kPa ( $P < 0.001$ ) and by ARFI from  $1.73 \pm 0.51$  m/sec to  $1.56 \pm 0.70$  m/s ( $P < 0.001$ ). In intrahepatic cholestasis, the mean value of LS changed from  $19.12 \pm 15.31$  kPa to  $13.50 \pm 9.79$  kPa by TE ( $P < 0.001$ ) and from  $2.29 \pm 0.68$  m/sec to  $1.89 \pm 0.72$  m/s by ARFI ( $P < 0.001$ ). ARFI sensitivity and specificity were respectively 92.6% and 50% (AUROC = 0.72) for  $F \geq 2$  with a cut-off value of  $m 1.53$  m/s; 70.6% and 66.7% (AUROC = 0.72) for  $F \geq 3$  with a cut-off value of 1.77 m/s; and 90% and 100% (AUROC = 0.93) for  $F = 4$  with a cut-off value of 2.43 m/s.



**Conclusions:** This study confirms that increased LS in patients with cholestasis, may be reduced after treatment, implying that increased values are not solely due to liver fibrosis, but to temporarily increased elasticity.

**Disclosure of Interest:** None Declared

## Simultaneous deletion of tight junction proteins ZO-1 and ZO-2 in hepatocytes results in the desintegration of the intrahepatic biliary system

Noemi Van Hul<sup>1</sup>, Matias Caldez<sup>1</sup>, Jaya Kausalya<sup>2</sup>, Jianliang Xu<sup>2</sup>, Matthew Dewhurst<sup>1</sup>, Walter Hunziker<sup>2</sup>, Philipp Kaldis<sup>1</sup>

<sup>1</sup>Cell Division and Cancer, <sup>2</sup>Epithelial Cell Biology, Institute for Molecular and Cell Biology, A\*STAR, Singapore, Singapore

Corresponding author's email: noemivanhul@yahoo.com

**Introduction:** Tight junctions (TJ) between adjacent hepatocytes form the only barrier preventing the regurgitation of bile salts into the sinusoidal blood flow. TJ adaptor proteins ZO-1 and ZO-2 not only connect transmembrane TJ proteins to the actin cytoskeleton but are also implicated in signaling pathways.

**Aims:** To better understand the multitasking of ZO-1 and ZO-2 in the liver ZO-1<sup>flox/flox</sup> ZO-2<sup>flox/flox</sup> ALB-Cre (or DKO) mice were generated.

**Results:** Three-month-old DKO mice suffer from hepatomegaly and jaundice. Blood values displayed strongly elevated levels of ALT, alkaline phosphatase and bilirubin indicating liver damage and bile spill over in DKO mice. Staining for CK19 revealed the degradation of the intrahepatic biliary system: when counting the number of bile duct epithelial cells (BECs) per transversally cut bile duct (BD), we noted a decrease in ZO-1/ZO-2 deleted livers (0.49 BECS/mm BD  $\pm$  0.016 vs 0.71  $\pm$  0.14 in WT livers). BECs in DKO livers were no longer observed as cuboidal cells organized in a uniform and continuous ring, but were found flattened while detaching from the underlining basement membrane resulting in the loss of lumen formation.

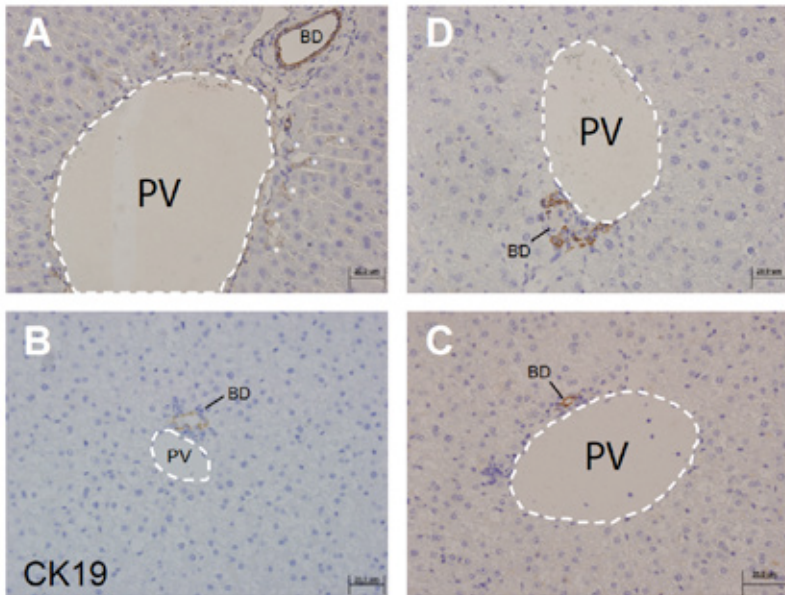
Where the WT BD diameter was consistently close to 25% of the portal vein (PV) diameter (panel A), BD were not proportionate to their associated PV in the DKO livers (45.06 %  $\pm$  11.24 for PV  $\leq$ 50 mm; 7.18%  $\pm$  1.36 for PV  $\geq$ 50mm) (Panel B-C). In addition, isolated liver progenitor cells (LPCs), described to reside near the PV in resting livers, were missing in DKO mice (0.15 LPCs/PV  $\pm$  0.21 vs. 24.55 LPCs/PV  $\pm$  5.30 in WT) (\* in Panel A vs. Panel B-C). Phalloidin, uniform and continuous along the WT canalicular margins, showed variations in staining intensity and distinct discontinuities in DKO

mice, confirming the junctional disruption. At 6 months of age, the biliary system was completely missing and DKO mice died around 8 months of age.

Simultaneous deletion of ZO-1 and ZO-2 by injection of AAV8-TBG-Cre into ZO-1<sup>flx/flx</sup> ZO-2<sup>flx/flx</sup> mice (no cre) and collection of the livers 2 months later, demonstrated similar BD degradation (Panel D), implying that the deletion of ZO-1 and ZO-2 in hepatocytes led to ductopenia.

**Conclusions:** In this DKO model, BECs are clearly a target of injury and although cholestasis is known to trigger a proliferative response of the biliary tree, we did not observe an attempt to compensate by the cholangiocytes nor a rescue by the LPCs. Identification of the components and pathways implicated is ongoing.

**Figure:**



**Disclosure of Interest:** None Declared

## Sensitive detection of cholangiocarcinoma using DNA methylation biomarkers in bile

**Kim Andresen<sup>1,2</sup>, Trine Folseraas<sup>\*3,4</sup>, Heidi Pharo<sup>1,2</sup>, Tom H. Karlsen<sup>3,4,5</sup>, Vemund Paulsen<sup>4</sup>, Marine Jeanmougin<sup>1,2</sup>, Kirsten M. Boberg<sup>3,4,5</sup>, Lars Aabakken<sup>4,5</sup>, Guro E. Lind<sup>1,2</sup>**

<sup>1</sup>Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospital, The Norwegian Radium Hospital, <sup>2</sup>Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, <sup>3</sup>Norwegian PSC Research Center, Department of Transplantation Medicine, <sup>4</sup>Section of Gastroenterology, Department of Transplantation Medicine, Division of Surgery, Inflammatory Medicine and Transplantation, Oslo University Hospital Rikshospitalet, <sup>5</sup>Institute for Clinical Medicine, University of Oslo, Oslo, Norway

**Corresponding author's email:** trine.folseraas@medisin.uio.no

**Introduction:** Cholangiocarcinoma (CCA) complicates primary sclerosing cholangitis (PSC) in 15-20% of cases. Lack of accurate diagnostic markers account for late diagnosis of CCA in PSC, and the majority of patients are diagnosed at an advanced, incurable stage of disease. Novel methods for early detection of- and firmly diagnosing PSC-CCAs are needed to qualify more patients for curative surgery

**Aims:** We aimed to establish a robust DNA methylation biomarker panel in bile that could diagnose CCA at early stage and with improved accuracy compared to conventional diagnostic methods.

**Material and Methods:** We have previously reported a promising DNA methylation biomarker panel for CCA detection in biliary brush samples, consisting of *CDO1*, *CNRIP1*, *SEPT9* and *VIM*, which outperformed the diagnostic accuracy of conventional brush cytology (Andresen et al, Hepatology, 2015). In the present study we derived ~300 bile samples from a total of 247 patients with CCA and other non-malignant liver diseases undergoing endoscopic retrograde cholangiopancreatography or liver transplantation. We have so far evaluated the performance of the four-gene DNA methylation panel in a selection of 92 bile samples using absolute quantitative droplet digital PCR technology. The preliminary bile series included n=15 patients with CCA (n=9 PSC-CCAs, n=6 non-PSC CCAs) and n=46 PSC patients without CCA development at time of bile sampling.



**Results:** Twelve among 15 (80%) of the CCAs were detected with 100% specificity, resulting in an area under the receiver operating characteristics curve of 0,965 (Asymp. Sig. 1,71E-8; Figure). For n=42 of the patients serial bile samples have been collected, making it possible to follow the biomarker panel status of these longitudinally at potential premalignant- and early stages of CCA. This is work in progress.

**Conclusions:** By using highly sensitive digital droplet technology and a robust DNA methylation biomarker panel, CCA can be accurately diagnosed in small volumes of bile.

**Figure:**

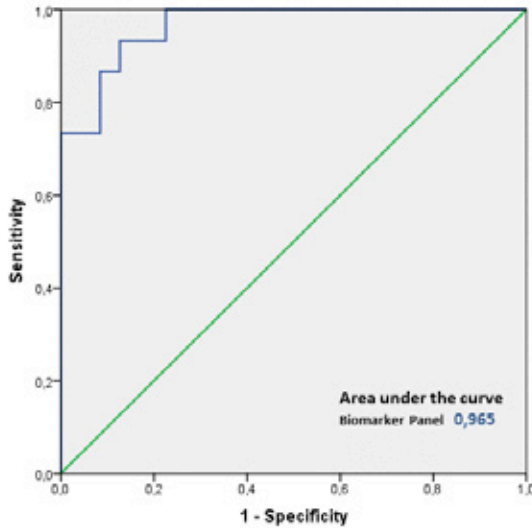


Figure: ROC curve of the combined biomarker panel

**Disclosure of Interest:** None Declared

## A familial syndrome of primary sclerosing cholangitis with a heterozygous germline mutation in SEMA4D

Xiaojun Jiang<sup>\* 1</sup>, Britt Sabina Petersen<sup>2</sup>, Geetha Venkatesh<sup>2</sup>, Andre Franke<sup>2</sup>, Annika Bergquist<sup>3</sup>, Tom Hemming Karlsen<sup>1</sup>, Niklas Björkström<sup>3</sup>, Espen Melum<sup>1</sup>

<sup>1</sup>Norwegian PSC Research Center, Department of Transplantation Medicine, Division of Cancer Medicine, Surgery and Transplantation, Oslo University Hospital Rikshospitalet, Oslo, Norway, <sup>2</sup>Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany, <sup>3</sup>Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

**Corresponding author's email:** [jiang.xiaojun@medisin.uio.no](mailto:jiang.xiaojun@medisin.uio.no)


**Introduction:** Primary sclerosing cholangitis (PSC) is an immune related cholestatic disease with multifactorial aetiology determined by genetic and environmental factors. Recent genome-wide association studies have revealed a series of genetic susceptibility loci in large case-control cohorts, but the molecular mechanism of PSC progression is still largely unknown.

**Aims:** Here we describe the first monogenetic form of PSC in a large Swedish family.

**Material and Methods:** Patient samples and controls were subjected to whole-exome sequencing. Peripheral blood mononuclear cells (PBMCs) were isolated and used for immune-phenotyping and lymphocyte purification. RNAseq was then done on the purified immune cells. Sequence reads were mapped to reference transcriptome TopHat. Gene level abundance was estimated by HTSeq and batch effects were removed by DESeq. Comparisons of the assigned groups were performed using R and DESeq. Pathway analysis was conducted using Consensus-Path-DB database.

**Results:** The Swedish family includes 5 individuals presenting with typical PSC following an autosomal dominant inheritance pattern. We identified a heterozygous missense mutation in the c-tail of semaphorin 4D (SEMA4D) in all patients but not in any of the healthy family members. Successful expression of mutated gene was confirmed in affected individuals. Further data revealed that the mutation gave the same amount of protein expression, membrane localization and extracellular domain shedding. Patients carrying the mutation possessed a normal immune cell distribution in peripheral blood, but decreased circulating Tregs. The expression profile of mutated T cells was dramatically





altered with a significant change in cell cycle pathway and related to PSC risk genes including SOCS1, PUS10 and REL.

**Conclusions:** We identified a novel mutation in SEMA4D that is a potential cause of a familiar syndrome of PSC.

**Disclosure of Interest:** None Declared

## NAD<sup>+</sup>/NADH redox status represents an abridged metabolic index that regulates intrinsic apoptosis in human cholangiocytes

Jung-Chin Chang<sup>\*1</sup>, Simei Go<sup>1</sup>, Ulrich Beuers<sup>1</sup>, Arthur Verhoeven<sup>1</sup>, Ronald Oude Elferink<sup>1</sup>

<sup>1</sup>Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Corresponding author's email: j.chang@amc.uva.nl

**Introduction:** The down-regulation of anion exchanger 2 (AE2) is increasingly recognized as a pathogenic feature of primary biliary cholangitis (PBC). The bicarbonate-responsive soluble adenylyl cyclase (sAC, ADCY10) is an evolutionarily conserved metabolic sensor that have been shown to regulate intrinsic apoptosis in various models. Using the human cholangiocyte cell line H69 (H69 cholangiocyte), we previously reported that down-regulation of AE2 led to intracellular alkalinization and up-regulated sAC expression, which sensitizes AE2-deficient H69 cholangiocytes to bile salt-induced apoptosis. We recently found that inhibition of sAC increased lactate secretion but reduced pyruvate secretion. In the presence of abundant lactate dehydrogenases, this increased lactate-to-pyruvate ratio following sAC inhibition is readily translated into a more reduced redox state of cytosolic NAD<sup>+</sup>/NADH, which could affect the activity of SIRT2, a cytosolic NAD<sup>+</sup>-dependent deacetylase.

**Aims:** To investigate if the cytosolic NAD<sup>+</sup>/NADH redox state and SIRT2 can modulate bile salt-induced apoptosis.

**Material and Methods:** Chenodeoxycholate-induced apoptosis in H69 cholangiocytes and rapitinal-induced apoptosis in the HepG2 hepatoma cell line were used to test the hypothesis. Cytosolic NAD<sup>+</sup>/NADH redox state was clamped by media of different lactate-to-pyruvate ratios. Apoptosis was monitored by enzymatic activity of caspase 3/7.

**Results:** Bile salt-induced apoptosis in H69 cholangiocytes was suppressed by a more reduced cytosolic NAD<sup>+</sup>/NADH redox state while strongly promoted by a more oxidized cytosolic NAD<sup>+</sup>/NADH redox state. Both the broad-spectrum sirtuin inhibitor EX527 and the SIRT2-specific inhibitor SirReal2 prevented the enhanced apoptosis under oxidized NAD<sup>+</sup>/NADH redox state. In addition, pre-treatment with octanoate, which

counteracted deacetylation by generating acetyl-CoA, also prevented bile salt-induced apoptosis. Similar results were obtained in the HepG2 hepatoma cell line.

**Conclusions:** We demonstrate that the cytosolic  $\text{NAD}^+/\text{NADH}$  redox status modulates intrinsic apoptosis in human H69 cholangiocytes and HepG2 cells. Both inhibition of SIRT2 and enhanced protein acetylation by octanoate protect against apoptosis, indicating that SIRT2 could be the  $\text{NAD}^+/\text{NADH}$  redox sensor and that an acetylation target is at play. Our result suggests that reduced AE2 expression stimulates the sAC- $\text{NAD}^+/\text{NADH}$ -SIRT2 axis, which can be exploited as a therapeutic target to reduce apoptosis in human cholangiopathies.

**Disclosure of Interest:** None Declared

## Whole transcriptome analysis of ductular reaction from patients with alcoholic hepatitis. Similarities to ductular reaction in DDC mouse model

Beatriz Aguilar<sup>\* 1, 2, 3</sup>, Daniel Rodrigo-Torres<sup>1, 2, 3</sup>, Mar Coll<sup>1, 2, 3</sup>, Lluís Revilla<sup>1, 2, 3</sup>, Delia Blaya<sup>1, 2, 3</sup>, Luis Perea<sup>1, 2, 3</sup>, Júlia Vallverdú<sup>1, 2, 3</sup>, Isabel Graupera<sup>1, 2, 3</sup>, Laurent Dubuquoy<sup>4</sup>, Philippe Mathurin<sup>4</sup>, Joan Caballeria<sup>1, 2, 3</sup>, Pere Gines<sup>1, 2, 3</sup>, Pau Sancho-Bru<sup>1, 2, 3</sup>

<sup>1</sup>IDIBAPS, <sup>2</sup>Liver Unit- Hospital Clínic, <sup>3</sup>Ciberehd, Barcelona, Spain, <sup>4</sup>Service des Maladies de l'appareil digestif et de la nutrition, Hôpital Huriez, Lille, France


**Corresponding author's email:** aguilar@clinic.cat

**Introduction:** Alcoholic hepatitis (AH) is characterized by the expansion of ductular reaction (DR), and the expression of liver progenitor cells (LPC) markers correlate with bad outcome. However, the gene expression profile of DR and its weight in disease progression is unknown. Moreover, no animal models of alcoholic liver disease reproduce the main characteristics of AH, particularly the DR expansion.

**Aims:** The aim of this study was to identify a gene set signature of the DR and to determine the best animal model to study DR expansion.

**Material and Methods:** KRT7+ cells were isolated from liver biopsies (n = 6) of patients with alcoholic hepatitis by laser capture microdissection and analyzed by next generation sequencing. Functional analysis was performed by Ingenuity Pathway Analysis. A transcriptomic signature comprising the most up-regulated 92 genes in KRT7+ cells was defined. Its expression was validated in data sets of patients with different stages of ALD (Alcoholic liver disease) by gene set enrichment analysis (GSEA). Expression profile was compared with FACS sorted LPC from two well-known animal models of DR expansion (DDC and CDE diet).

**Results:** Transcriptomic analysis showed an up-regulation of 2345 genes in KRT7+ cells as compared to negative fraction. Expression of LPC markers (HNF1 $\beta$ , KRT7, EpCAM, PROM1) was confirmed by qPCR and immunohistochemistry. As expected, GSEA showed a high overlap of microdissected signature in patients with AH and a positive correlation with ALD progression. At a functional level, KRT7+ showed enrichment in genes involved in tight junction signaling and cell adhesion, cell death pathway and



regulation of autophagy. Next, in order to find the best mouse model reproducing the DR present in AH, AH transcriptomic signature was compared to DDC and CDE LPC expression profile. Interestingly, GSEA revealed that DR signature from AH patients show a higher degree of similarity with the gene expression profile of cells derived from DDC model, which has been reported to show no contribution of LPC to hepatocyte regeneration.

**Conclusions:** Here we report the gene expression signature of DR in AH. Although, the gene expression profile is highly enriched in LPCs markers, the high similarity to DDC profile may suggest a poor contribution to hepatocyte regeneration. This study suggests that DDC mouse model may be a useful tool to mimic DR in AH.

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## TWEAK/Fn14 signalling drives chemokine secretion in cholangiocarcinoma

**Benjamin J. Dwyer<sup>1,2</sup>, Luke Boulter<sup>1,3</sup>, Rachel Guest<sup>1</sup>, Eoghan O'Duibhir<sup>1</sup>, Tak Yung Man<sup>1</sup>, Davina Wojtacha<sup>1</sup>, Lara Campana<sup>1</sup>, Philip Starkey-Lewis<sup>1</sup>, John Olynyk<sup>2,4,5</sup>, Grant Ramm<sup>6,7</sup>, Janina Tirnitz-Parker<sup>2,8</sup>, Stuart Forbes<sup>1</sup>**

<sup>1</sup>MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, United Kingdom, <sup>2</sup>School of Biomedical Sciences, CHIRI Biosciences Research Precinct, Curtin University, Perth, Australia, <sup>3</sup>MRC Human Genetics Unit, Western General Hospital Campus, Edinburgh, United Kingdom, <sup>4</sup>Department of Gastroenterology, Fremantle Hospital, Fremantle, <sup>5</sup>Institute for Immunology and Infectious Diseases, Murdoch University, Perth, <sup>6</sup>Faculty of Health Sciences, University of Queensland, <sup>7</sup>QIMR Berghofer Medical Research Institute, Brisbane, <sup>8</sup>School of Medicine and Pharmacology, University of Western Australia, Perth, Australia

**Corresponding author's email:** [bdwyer@exseed.ed.ac.uk](mailto:bdwyer@exseed.ed.ac.uk)


**Introduction:** Cholangiocarcinoma (CC) is typically detected at an advanced stage and rarely successfully treated by resection or chemotherapy. Tumour associated macrophages provide critical mitogenic factors, such as Wnt ligands, to drive CC growth. Another macrophage-derived signal, tumour necrosis factor-like weak inducer of apoptosis (TWEAK), drives the ductular reaction in chronic liver disease. TWEAK binds to its receptor, fibroblast growth factor-inducible 14 (Fn14), on the surface of cholangiocytes to drive proliferation via NF- $\kappa$ B. Although a mitogen for liver progenitor and ductal cells, TWEAK/Fn14 signalling has not been evaluated in CC.

### **Aims:**

- 1) To determine if the components of the TWEAK/Fn14 pathway are expressed in CC.
- 2) To define the function of TWEAK/Fn14 signalling in CC.
- 3) To test whether this pathway can be therapeutically targeted.

**Material and Methods:** TWEAK signalling was assessed in human tissue, thioacetamide-induced mouse and rat CC, and human CC cell lines by immunostaining, western blotting, flow cytometry, protein immunoassay and qPCR.

**Results:** TWEAK and Fn14 are significantly upregulated in CC tissues from human, rat and mouse, and a subset of liver cells upregulate Fn14 protein early in CC development in rats. We found that TWEAK activates both canonical and non-canonical NF- $\kappa$ B



signalling and drives the secretion of several key chemokines involved in inflammatory cell chemotaxis *in vitro*. One of these TWEAK-responsive chemokines, MCP-1, was expressed in CC cells of mouse, rat and human origin. Furthermore, antibody blocking of MCP-1 reduced CC xenograft size *in vivo*.

**Conclusions:** We have demonstrated that TWEAK/Fn14 pathway components are significantly upregulated in CC in rodent disease models and clinical samples. Our cell line studies potentiate a role for TWEAK/Fn14 signalling to drive inflammatory cell recruitment to the CC niche, and targeting TWEAK-inducible MCP-1 may provide therapeutic benefit in CC.

**Disclosure of Interest:** None Declared

## Type I interferons are sensitive inflammatory biomarkers in primary sclerosing cholangitis

Christina Mehrfeld<sup>1</sup>, Marcin Krawczyk<sup>1,2</sup>, Frank Lammert<sup>1</sup>, Piotr Milkiewicz<sup>3,4</sup>, Mirosław Kornek<sup>1</sup>, Veronika Lukacs-Kornek<sup>1</sup>

<sup>1</sup>Internal Medicine II, University of Saarland, Homburg, Germany, <sup>2</sup>Department of General, Transplant and Liver Surgery, <sup>3</sup>Liver and Internal Medicine Unit, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Warsaw, <sup>4</sup>Translational Medicine Group, Pomeranian Medical University, Szczecin, Poland

Corresponding author's email: lukacsver@aol.com


**Introduction:** Primary sclerosing cholangitis (PSC) is an idiopathic, heterogeneous cholestatic liver disease that is characterized by progressive biliary inflammation and bile duct destruction that progresses to fibrosis and end-stage liver disease. While the exact pathomechanism is complex and not entirely understood, PSC is associated with immune cell infiltration and inflammatory activation of cholangiocytes.

**Aims:** We aimed to analyze novel inflammatory pathways previously associated with various autoimmune/inflammatory disorders and looked for their correlation with PSC disease activity.

**Material and Methods:** Liver serum samples from patients with primary sclerosing cholangitis (n=27) and healthy individuals (n=16) were tested for routine clinical parameters (such as alanine transaminase /ALT, alkaline phosphatase/ALP, bilirubin) and circulating inflammatory molecules that indicate immune cell activation. Using Elisa and cell-based reporter system, type I interferons and soluble OX40L were determined.

**Results:** Patient with PSC failed to show alteration in serum soluble OX40L level ( $2.908 \pm 0.4878$  ng/ml) compared to healthy individuals ( $2.144 \pm 0.3374$  ng/ml). However, type I IFN increased 1.6-fold ( $p < 0.0001$ ) in PSC patients compared to healthy individuals (AUROC = 0.9213,  $p < 0.0001$ ; sensitivity = 88.89% and specificity = 93.75%, PPV = 96% and NPV = 83.3%) and closely correlated with serum ALP values ( $r = 0.8558$ ,  $p < 0.0001$ ). IFN levels showed poor correlation with ALT ( $r = 0.5556$ ,  $p = 0.0026$ ), serum bilirubin ( $r = 0.2147$ ,  $p = 0.2823$ ) and with other inflammatory parameters such as white blood cells ( $r = 0.4681$ ,  $p = 0.0138$ ) and CRP ( $r = 0.3661$ ,  $p = 0.0603$ ).





**Conclusions:** Our data demonstrate that in PSC type I IFNs are closely associated with biliary damage and likely represent a prominent inflammatory pathway in this liver disorder.

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## SOX17 selectively sensitizes cholangiocarcinoma cells to anticancer drugs by interfering with promoter activation of the export pumps ABCC3 and ABCG2

Elisa Lozano<sup>\* 1, 2</sup>, Oscar Briz<sup>1, 2</sup>, Maitane Asensio<sup>1</sup>, Laura Perez-Silva<sup>1</sup>, Maria Jesus Monte<sup>1, 2</sup>, Maria Jose Perez<sup>1, 2</sup>, Elisa Herraiz<sup>1, 2</sup>, Marta R. Romero<sup>1, 2</sup>, Maria Angeles Serrano<sup>1, 2</sup>, Rocio Isabel R. Macias<sup>1, 2</sup>, Luis Bujanda<sup>2, 3</sup>, Jesus M. Banales<sup>2, 3</sup>, Jose Juan G. Marin<sup>1, 2</sup>


<sup>1</sup>Experimental Hepatology and Drug Targeting (HEVEFARM), IBSAL, University of Salamanca, Salamanca, <sup>2</sup>National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Carlos III National Health Institute, Madrid, <sup>3</sup>Department of Hepatology and Gastroenterology. Biodonostia Biomedical Research Institute. San Sebastian University Hospital. Basque Country University., San Sebastian, Spain

**Corresponding author's email:** elisa\_bilogia@usal.es

**Introduction:** The prognosis of cholangiocarcinoma (CCA) is often fatal due in part to its poor response to chemotherapy. Among several mechanisms of chemoresistance (MOC), the reduced intracellular concentration of anticancer drugs plays an important role. This is mainly due to active drug export through ATP-binding cassette (ABC) proteins, such as ABCG2 and ABCC3. SOX17, a transcription factor that inhibits Wnt/ $\beta$ -catenin pathway has been reported to be downregulated in CCA. Moreover, restoration of SOX17 expression in CCA cells results in tumour suppression.

**Aims:** Here we have investigated whether SOX17 expression may also affect CCA sensitivity to chemotherapy.

**Material and Methods:** Viral vectors containing SOX17 ORF were generated to transduce CCA cells (EGI-1 and TFK-1). Cell viability in response to incubation with commonly used anti-CCA drugs was determined by MTT test. Taqman Low Density Arrays (TLDA) were designed to measure mRNA abundance of  $\approx 100$  genes involved in several MOCs. Single RT-qPCR, WB and IF were used to evaluate gene expression. Export activity of ABC pumps was determined by flow cytometry using appropriate fluorescent substrates. Firefly luciferase (Luc2) was fused to ABC promoters to carry out promoter-reporter assays. Mouse xenograft model was used for *in vivo* evaluation of chemotherapy efficacy.



**Results:** SOX17 overexpression selectively enhanced the cytostatic response of CCA cells to SN-38, 5-FU and mitoxantrone, but not to gemcitabine or cisplatin. The magnitude of chemosensitization was dependent on SOX17 expression levels (MOI25 >MOI3). The analysis of changes in MOC gene expression profile revealed that SOX17 overexpression affected several of these genes, mainly those encoding ABC proteins. Thus, a significant reduction in ABCC3/ABCG2 expression was found. Interference with ABC gene promoter activity seems to be involved in the mechanism of SOX17-induced ABCC3/ABCG2 downregulation. Functional studies supported a reduced ability of SOX17-overexpressing CCA cells to export specific substrates of these pumps. Moreover, combined ABCG2/ABCC3 substrate specificity matched the observed selective chemosensitization. SOX17-induced better response to ABCG2/ABCC3 substrates was confirmed by *in vivo* experiments.

**Conclusions:** In addition to the tumour suppression role of SOX17, its forced expression induces selective chemosensitization due to downregulation of some ABC proteins and reduction in the ability of CCA cells to export anticancer drugs.

**Disclosure of Interest:** None Declared

## Alagille syndrome results in loss of polarity of cholangiocytes, rather than cholangiocyte loss per se

Emma R. Andersson\*<sup>1</sup>, Simona Hankeova<sup>1</sup>, Indira Chivukula<sup>1</sup>, Antal Nemeth<sup>1</sup>, Björn Fischler<sup>1</sup>, Daniel Ramsköld<sup>2</sup>, Helena Storrvall<sup>2</sup>, Yat Long Tsoi<sup>2</sup>, Kenneth Chien<sup>2</sup>, Rikard Sandberg<sup>2</sup>, Vitezslav Bryja<sup>3</sup>, Urban Lendahl<sup>2</sup>

<sup>1</sup>Karolinska Institutet, Huddinge, <sup>2</sup>Karolinska Institutet, Stockholm, Sweden, <sup>3</sup>Masaryk University, Brno, Czech Republic

**Corresponding author's email:** emma.andersson@ki.se

**Introduction:** Alagille syndrome is a rare autosomal dominant genetic disorder caused by mutations in *JAG1* or in *NOTCH2*, and is characterised by, among other symptoms, cholestasis and bile duct paucity.

**Aims:** It is currently unclear whether abrogated Notch signaling leads to fewer biliary cells, or whether bile duct morphogenesis is specifically disturbed in Alagille patients. We aimed to determine whether Alagille patients generally express less bile duct-specific transcripts, or whether patients mis-express a subset of genes relevant to biliary cells.

**Material and Methods:** We performed genome-wide transcriptome studies of biopsies from livers from Alagille patients, and from a mouse model for Alagille syndrome. In order to identify novel markers for cholangiocytes we cross-referenced the transcriptome data with protein expression patterns from the Human Protein Atlas (HPA, <http://www.proteinatlas.org>), a tissue-based map of the human proteome.

**Results:** Comparison of the bile duct-enriched proteins from HPA to the gene set dysregulated in Alagille patients revealed 8 upregulated, and 18 downregulated, novel bile duct markers in Alagille syndrome. The most highly downregulated biliary genes encoded proteins localized at the apical surface of bile ducts.

In contrast, there was no downregulation of genes encoding biliary cells in general.

**Conclusions:** Biliary defects in Alagille patients are more likely caused by morphogenesis and polarity defects than by differentiation defects.

**Disclosure of Interest:** None Declared

## Protein kinase CK2 as potential therapeutic target in cholangiocarcinoma

**Giovanni Di Maira<sup>1</sup>, Alessandra Gentilini<sup>1</sup>, Elisabetta Rovida<sup>2</sup>, Mauro Salvi<sup>3</sup>, Christian Borgo<sup>3</sup>, Daniele Ottaviani<sup>3</sup>, Maria Ruzzene<sup>3</sup>, Jesper Andersen<sup>4</sup>, Chiara Raggi<sup>1</sup>, Fabio Marra<sup>1</sup>**

<sup>1</sup>Medicina Sperimentale e Clinica, <sup>2</sup>Oncologia Sperimentale, University of Florence, Florence, <sup>3</sup>Scienze Biomediche, University of Padova, Padova, Italy, <sup>4</sup>Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Copenhagen, Denmark

**Corresponding author's email:** giovanni.dimaira@unifi.it

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**Introduction:** Cholangiocarcinoma (CCA) is a form of cancer that arise from cholangiocytes in the biliary tree and it accounts for approximately 10–25% of all hepatobiliary malignancies. The mechanisms underlying the CCA occurrence and progression are largely unknown. CK2 is a ubiquitous serine-threonine protein kinase composed of two catalytic subunits ( $\alpha$  and/or  $\alpha'$ ) and two regulatory subunits ( $\beta$ ), which contributes to the malignant phenotype in various types of cancer and its overexpression is associated with unfavorable prognosis. CK2 may be targeted by different inhibitors, including CX-4945, that is currently being evaluated in clinical trials.

**Aims:** The aim of this study is to understand the involvement of CK2 in CCA biology and evaluate the potential therapeutic relevance of targeting CK2 in CCA.

**Material and Methods:** Transcriptomic profiling of 104 cholangiocarcinoma samples was performed by microarray analysis. CX4945 was used as a specific CK2 inhibitor. Catalytic subunit of CK2 was stably silenced using CRISPR/Cas9 genome editing technology. CK2 activity was measured using a CK2-specific peptide substrate. Cell cycle progression was analyzed using flow cytometry. Cell migration and cell invasion assays were performed using *in vitro* scratch method and boyden chamber.

**Results:** First we analyzed CK2 expression in human CCA samples by transcriptomic analysis and we found that CK2 subunits expression was significantly increased compared to surrounding matched normal tissues.

CK2 expression and catalytic activity was found elevated in CCA cells compared to intrahepatic biliary epithelial cells. Inhibition of CK2 decreased cell viability and activated

apoptosis. Brdu assay and FACS analysis showed that CK2 is necessary for CCA cell proliferation, in particular CK2 is involved in the regulation of G1/S transition. This finding was further confirmed by wb analysis which showed an altered expression of key regulators of cell cycle progression such as Cyclin E and p27Kip in CCA cells treated with CK2-inhibitor. Finally CK2 was involved in the modulation of the protumorigenic properties of CCA cells, in fact knock-down of CK2 activity reduces cell motility, invasiveness and increases sensitivity to chemotherapeutic drugs.

**Conclusions:** Considering the role of CK2 in the modulation of malignant phenotype of CCA cells through the regulation of pivotal cellular processes such as proliferation, cell survival, chemotaxis and invasion, CK2 may represent a promising therapeutic target of CCA which deserves further investigation.

**Disclosure of Interest:** None Declared

## Defects in protein processing may underlie the development of hepatic cysts in patients with polycystic liver disease

**Liyanne F. M. van de Laarschot<sup>\*1</sup>, Sanne A. B. Hermesen<sup>1</sup>, Titus F. Wijnants<sup>1</sup>, Edgar S. Wills<sup>1</sup>, Hans J. Wessels<sup>2</sup>, Joost P. H. Drenth<sup>1</sup>**

<sup>1</sup>Gastroenterology and Hepatology, <sup>2</sup>Radboud Proteomics Center, Translational Metabolic Laboratory, Department of Laboratory Medicine, RADBOUDUMC, Nijmegen, The Netherlands

**Corresponding author's email:** [liyanne.vandelaarschot@radboudumc.nl](mailto:liyanne.vandelaarschot@radboudumc.nl)

**Introduction:** Mutations in *PRKCSH* and *SEC63* cause autosomal dominant polycystic liver disease. Both are involved in co-translational protein translocation and maturation of glycoproteins in the endoplasmic reticulum. Defects of either gene will have a significant impact on the biogenesis of polycystic liver disease (PLD) associated proteins.

**Aims:** We aimed to identify which proteins are affected in PLD.

**Material and Methods:** Organoids cultured from PLD patients with and without known mutations were used for RNA sequencing. Heatmaps were generated with gene subsets created from the lists of significant genes. Functional analysis of identified genes was performed with Ingenuity Pathway Analysis. Hepatic cyst fluid from PLD patients who underwent aspiration-sclerotherapy or cyst fenestration was obtained for glycopeptides profiling using a mass-spectrometry based technique.

**Results:** We isolated RNA from organoids of 8 PLD patients (3 *PRKCSH* mutants) and 4 patients without known liver histopathology. Preliminary RNAseq data analysis revealed distinct gene expression profiles of organoids cultured from PLD patients with *PRKCSH* mutation, patients without mutations and normal patients. PLD organoids displayed a heterogeneous expression pattern within their group. *PRKCSH* mutants shared expression of a subset of genes. The changes indicate endoplasmic reticulum related processes, such as 'protein folding in endoplasmic reticulum' and 'response to endoplasmic reticulum stress'. Mass-spectrometry analysis distinguishes glycopeptide profiles from cyst fluid of PLD patients with ( $n=4$ ) and without ( $n=8$ ) *PRKCSH* mutation.

**Conclusions:** These data strongly suggest a specific defect in protein maturation and localization in the endoplasmic reticulum, and protein glycosylation play a role in the development of hepatic cysts in PLD patients.

**Disclosure of Interest:** None Declared



## The CDE-induced liver injury causes impaired canalicular bile flow and expansion of small biliary branches that connect to intralobular canaliculi to drain bile

Laure-Alix Clerbaux<sup>\* 1</sup>, Rita Manco<sup>1</sup>, Noémi Van Hul<sup>2</sup>, Régina Español-Suñer<sup>3</sup>, Caroline Bouzin<sup>4</sup>, Christine Sempoux<sup>5</sup>, Neil Theise<sup>6</sup>, Isabelle Leclercq<sup>1</sup>

<sup>1</sup>Laboratory of Hepatogastroenterology, Université Catholique de Louvain, Brussels, Belgium,

<sup>2</sup>Cell division and cancer laboratory, A\*STAR, Singapore, Singapore, <sup>3</sup>University of California, San Francisco, United States, <sup>4</sup>Université Catholique de Louvain, Brussels, Belgium, <sup>5</sup>Division of Pathology, CHUV, Lausanne, Switzerland, <sup>6</sup>Mount Sinai Beth Israel medical center, New York, United States

**Corresponding author's email:** laure-alix.clerbaux@uclouvain.be

**Introduction:** Ductular reaction (DR), which refers to expansion from the portal area of immature-like cells with biliary phenotype, are observed in many liver diseases in both humans and rodents. DR cells are also called liver progenitor cells (LPC) as they have been shown to differentiate into both hepatocytes and cholangiocytes in culture, suggesting that DR/LPC are activated following liver injury and generate a reservoir to restore hepatic cell loss. However, so far, only a very small number of LPC-derived hepatocytes were observed *in vivo* and only in the model of choline-deficient ethionine-supplemented (CDE) diet.

**Aims:** This observation prompted us to investigate the pathophysiological role of DR *in vivo* in the CDE model. We propose that CDE injury impairs canalicular bile flow and that DR/LPC expand into the parenchyma as biliary structures to connect bile canaliculi to bile ducts.

**Results:** First, following CDE diet, we observe reduced bile flow rate, higher bile acids levels in the serum, impaired hepatic uptake of bile acids, distortion of the pericanalicular network and disruption of the tight junction integrity at the apical pole of hepatocytes. All these data suggest that the canalicular bile flow and the blood-bile barrier are impaired in the livers of CDE-fed mice. In parallel, after CDE feeding, we observe progressive expansion of small biliary cells lining on a basement membrane and delineating a tubular lumen; that possess the machinery needed to sense bile and that connect to the intralobular canaliculi system.

**Conclusions:** Our results support that, in the CDE-model of liver injury, DR/LPC penetrate into the parenchyma as biliary tubular structure able to establish connection with the hepatocytes canaliculi system to ensure bile drainage in the injured liver.

**Disclosure of Interest:** None Declared

## High-throughput sequencing analysis of tissue-resident gut and liver B cells reveals antigen-driven clonal expansion in primary sclerosing cholangitis-inflammatory bowel disease

**Brian K. Chung**<sup>\* 1, 2, 3, 4</sup>, **Eva Kristine Klemsdal Henriksen**<sup>3, 4, 5</sup>, **Gideon M. Hirschfield**<sup>1, 2</sup>, **Tom H. Karlsen**<sup>3, 4, 5</sup>, **Evaggelia Liaskou**<sup>1, 2</sup>

<sup>1</sup>Centre for Liver Research and NIHR Birmingham Liver Biomedical Research Unit, Institute of Immunology and Immunotherapy, College of Medical and Dental Sciences, University of Birmingham, <sup>2</sup>Birmingham Health Partners, Institute of Translational Medicine, University Hospitals Birmingham, Birmingham, United Kingdom, <sup>3</sup>Norwegian PSC Research Center, Department of Transplantation Medicine, Division of Surgery, Inflammatory Medicine and Transplantation, Oslo University Hospital Rikshospitalet, <sup>4</sup>Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, <sup>5</sup>Research Institute of Internal Medicine, Division of Surgery, Inflammatory Medicine and Transplantation, Oslo University Hospital Rikshospitalet, Oslo, Norway

**Corresponding author's email:** [b.chung@bham.ac.uk](mailto:b.chung@bham.ac.uk)

**Introduction:** Primary sclerosing cholangitis (PSC) is an immune-mediated liver disorder featuring chronic bile duct inflammation complicated by biliary strictures, cirrhosis and end-stage liver disease. As 50-80% of PSC cases are associated with inflammatory bowel disease (PSC-IBD), we hypothesize that antigens shared within the gut-liver axis drive hepato-biliary pathology in PSC-IBD.

**Aims:** To assess antigen-driven expansion of gut and liver B cells in PSC-IBD and better understand the antigenic triggers and immuno-pathophysiology of PSC-IBD.

**Material and Methods:** To determine if shared gut-liver antigens promote B cell responses in PSC-IBD, we evaluated B-cell clonality among paired gut and liver patient samples using high-throughput deep sequencing of the B-cell receptor (BCR) immunoglobulin heavy chain (IGH; Adaptive Biotechnologies). Metadata analysis of the hypervariable complementarity-determining region 3 (CDR3) and IGH variable (IGHV) gene family usage was performed using ImmunoSeq.

**Results:** Using inflamed colon and liver tissue from PSC-IBD patients (n=10), we detected a significant enrichment of shared B-cell clonotypes in paired samples compared

to unpaired specimens (paired:  $345 \pm 77$ , unpaired:  $4 \pm 1$ ; mean  $\pm$  SEM,  $p < 0.0001$ ). Overlapping gut and liver clonotypes in paired samples were 4.27% of the total productive IGH sequences on average (range: 0.69 – 11.32%) and were present at higher frequencies in the liver than gut (9/10). Intriguingly, the top 4 clonotypes shared between gut and liver tissues were found in 5/10, 6/10 and 7/10 of patients and 3/4 clonotypes expressed considerable CDR3 amino acid homology (CXRD'TXR) suggesting these clonotypes may represent novel disease-associated B-cell clonotypes for PSC-IBD as none of the CDR3 sequences were found by metadata analysis. Evaluation of IGHV gene family usage showed preferential IGHV3-09 rearrangement by shared clonotypes whereas IGHV4-34 was over-represented in liver-restricted clonotypes.

**Conclusions:** A high proportion of tissue-resident gut and liver B cells appear to recognize similar or structurally-related antigens in PSC-IBD and exhibit preferential IGHV3-09 gene usage. These data support the concept of the gut-liver axis in PSC-IBD and suggest that pathogenic B cells originating in the gut may undergo clonal expansion in the liver of patients with PSC-IBD. Large-scale single-cell BCR sequencing efforts may further refine these observations and reveal greater insights into the triggering antigens of PSC-IBD.

**Disclosure of Interest:** None Declared

## The crucial role of KITENIN in cholangiocarcinogenesis

Khac Cuong Bui<sup>1, 2</sup>, Samarpita Barat<sup>1</sup>, Xi Chen<sup>1</sup>, Przemyslaw Bozko<sup>1</sup>, Mai Ly T. Nguyen<sup>1</sup>, Vikas Bhuria<sup>1</sup>, Jun Xing<sup>1</sup>, Toan Nguyen Linh<sup>2</sup>, Song Le Huu<sup>3</sup>, Thirumalaisamy P. Velavan<sup>4</sup>, Peter G. Kremsner<sup>4</sup>, Bence Sipos<sup>5</sup>, Ludwig Wilkens<sup>6</sup>, Nisar P. Malek<sup>1</sup>, Ruben R. Plentz<sup>1</sup>

<sup>1</sup>Department of Internal Medicine I, Medical University Hospital, Tuebingen, Germany,

<sup>2</sup>Department of Pathophysiology, Vietnam Military Medical University, <sup>3</sup>108 Military Central Hospital, Hanoi, Vietnam, <sup>4</sup>Institute of Tropical Medicine, <sup>5</sup>Institute of Pathology, Medical University Hospital, Tuebingen, <sup>6</sup>Institute of Pathology, Hannover Medical School, Hannover, Germany

**Corresponding author's email:** khac-cuong.bui@med.uni-tuebingen.de

**Introduction:** Cholangiocarcinoma (CC) is the second most common primary hepatic malignancy. Worldwide incidence and mortality rates are rising, but systemic treatments are limited. Kangai 1 C-terminal interacting tetraspanin (KITENIN), a member of the tetraspanin family, is expressed in different tumors, is important for tumor progression and metastasis. However, the role of KITENIN in cholangiocarcinogenesis is not clear yet.

**Aims:** To analyse the function of KITENIN in human cell lines (SZ-1, TFK-1), human CC tissues and in an engineered mouse model (Alb-Cre/KRAS<sup>G12D</sup>/p53<sup>L/L</sup>) of CC.

**Material and Methods:** Expression of KITENIN was determined by immunohistochemistry, immunofluorescence and Western Blot. We analysed the effect by small interfering RNA against KITENIN on cell proliferation and mobility by using proliferation-, migration- and invasion-assays. Western Blot was applied to measure the expression of epithelial-mesenchymal transition (EMT) markers.

**Results:** KITENIN is highly expressed in human CC cell lines (n=2), human CC (n=30) and murine CC (n=5). Silencing of KITENIN effectively reduced proliferation, migration and invasion in both intra- and extra-hepatic human CCC cells (p < 0.05). Down-regulation of KITENIN impaired the expression of EMT markers (N-cadherin, Vimentin, Slug and Snail).

**Conclusions:** Our data demonstrate that KITENIN plays an important role for cholangiocarcinogenesis. KITENIN might become a new and potential therapeutic target against human CC.

**Disclosure of Interest:** None Declared

## Identification and in silico characterization of six novel *GANAB* mutations in polycystic liver disease

Liyanne F. M. van de Laarschot<sup>\*1,1</sup>, René H. M. te Morsche<sup>1</sup>, Alexander Hoischen<sup>2</sup>, Wybrich R. Cnossen<sup>1</sup>, Hanka Venselaar<sup>3</sup>, Joost P. H. Drenth<sup>1</sup>

<sup>1</sup>Gastroenterology and Hepatology, <sup>2</sup>Human Genetics, <sup>3</sup>Centre for Molecular and Biomolecular Informatics, RADOUDUMC, Nijmegen, The Netherlands

**Corresponding author's email:** liyanne.vandelaarschot@radboudumc.nl

**Introduction:** Glucosidase II is part of the functional pathway of co-translational protein translocation and maturation in the endoplasmic reticulum. It is implicated in autosomal dominant polycystic liver disease (ADPLD) and autosomal dominant polycystic kidney disease (ADPKD). The  $\beta$ -subunit of glucosidase II, encoded by *PRKCSH*, has been identified as one of the causative genes of ADPLD. Recent data suggest that the  $\alpha$ -subunit of glucosidase II, encoded by *GANAB*, is associated with ADPKD and ADPLD.

**Aims:** We aimed to identify *GANAB* mutations in an independent cohort of patients with the primary phenotype of polycystic liver disease and to predict the influence of these mutations on glucosidase II function.

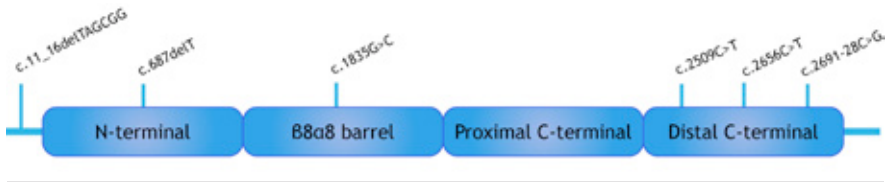
**Material and Methods:** We used molecular inversion probe (MIP) analysis to identify genetic mutations in *GANAB* in a cohort of patients with polycystic liver disease. Both patients with ADPKD and ADPLD were included for analysis. Mutations identified with MIP analysis were validated using Sanger sequencing. Bioinformatics prediction tools (PolyPhen-2, Align GVGD, SIFT, MutationTaster) were used to predict the functional significance of the mutations. YASARAandWHAT IF were used for analysis of the structural effects of the mutations.

**Results:** We identified and validated 6 new bona fide *GANAB* mutations in 7 unrelated families. These are 2 frameshift (c.687delT and c.11\_16delTAGCGG), 1 splicing (c.2691-28C >G), 2 nonsense (c.2509C >T and c.2656C >T) and 1 missense (c.1835G >C) mutation. In silico analysis showed c.687delT and c.11\_16delTAGCGG are located in N-terminal domain of the protein. These mutations probably lead to a total defective protein. c.1835G >C is located in the active site of the protein, the  $\beta_8\alpha_8$  barrel domain. It is predicted to disrupt the composition of the active site and reduce enzymatic activity. The remaining mutations (c.2691-28C >G, c.2509C >T and c.2656C >T) are located in

the distal C-terminal domain, where normally interaction with *PRKCSH* takes place. The mutations could result in early termination of this domain. It is speculated this disrupts the ability of the two subunits to interact.

**Conclusions:** We describe six novel *GANAB* mutations that can cause polycystic liver disease in a mixed population of ADPKD and ADPLD patients. These mutations are found in functionally important domains of  $\alpha$ -subunit of glucosidase II, which may lead to impaired enzymatic activity of the complex.

**Figure:**



**Disclosure of Interest:** None Declared



## MiRNA-506 promotes primary biliary cholangitis (PBC)-like features in cholangiocytes and immune activation

Oihane Erice\*<sup>1</sup>, Patricia Munoz-Garrido<sup>1, 2</sup>, Javier Vaquero<sup>3</sup>, Maria Jesus Perugorria<sup>1,2,4</sup>, Maite G. Fernandez-Barrena<sup>2,5</sup>, Elena Saez<sup>5</sup>, Alvaro Santos-Laso<sup>1</sup>, Ander Arbelaz<sup>1</sup>, Raul Jimenez-Agüero<sup>1</sup>, Joaquin Fernandez-Irigoyen<sup>6</sup>, Enrique Santamaria<sup>6</sup>, Veronica Torrano<sup>7, 8</sup>, Arkaitz Carracedo<sup>4, 7, 8, 9</sup>, Meenakshisundaram Ananthanarayanan<sup>10</sup>, Marco Marzioni<sup>11</sup>, Jesus Prieto<sup>2, 5</sup>, Ulrich Beuers<sup>12</sup>, Ronald O. Elferink<sup>12</sup>, Nicholas F. LaRusso<sup>13</sup>, Luis Bujanda<sup>1, 2</sup>, Jose Juan G. Marin<sup>2, 3</sup>, Jesus Maria Banales<sup>1, 2, 4</sup>

<sup>1</sup>Department of Liver and Gastrointestinal Diseases, Biodonostia Research Institute, San Sebastian, <sup>2</sup>National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Carlos III National Institute of Health, Madrid, <sup>3</sup>Experimental Hepatology and Drug Targeting (HEVEFARM), Biomedical Research Institute of Salamanca (IBSAL), Salamanca, <sup>4</sup>IKERBASQUE, Basque Foundation for Science, Bilbao, <sup>5</sup>Division of Hepatology, CIMA of the University of Navarra, <sup>6</sup>Proteored-ISCI, Proteomics Unit, Navarrabiomed, IdiSNA, Pamplona, <sup>7</sup>CIC bioGUNE, Derio, <sup>8</sup>CIBERONC, Carlos III National Institute of Health, Madrid, <sup>9</sup>Biochemistry and Molecular Biology Department, University of the Basque Country (UPV/EHU), Bilbao, Spain, <sup>10</sup>Liver Center, Yale University School of Medicine, New Haven, United States, <sup>11</sup>Department of Gastroenterology, Università Politecnica delle Marche, Ancona, Italy, <sup>12</sup>Gastroenterology and Hepatology, Tytgat Institute for Liver and Intestinal Research, Amsterdam, The Netherlands, <sup>13</sup>Gastroenterology and Hepatology, Mayo Clinic, Rochester, United States

**Corresponding author's email:** oihane.eric@biodonostia.org

**Introduction:** Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease associated with autoimmune phenomena targeting the intrahepatic bile duct cells (i.e. cholangiocytes). PBC etiopathogenesis remains obscure, but most of patients develop anti-mitochondrial auto-antibodies against the pyruvate dehydrogenase complex-E2 (PDC-E2). Recently, microRNA dysregulation was described in liver and immune cells of PBC patients but their functional relevance is mostly unknown. We previously reported that miR-506 is overexpressed in PBC cholangiocytes and directly targets the Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> anion exchanger 2 (AE2) and type III inositol 1,4,5-trisphosphate receptor (InsP3R3), leading to cholestasis.

**Aims:** The regulation of miR-506 gene expression as well as its role in cholangiocyte pathophysiology and immune activation was studied.

**Results:** Different pro-inflammatory cytokines found overexpressed in PBC livers [such as interleukins 8, 12, 17 and 18, as well as tumor necrosis factor alpha (TNF $\alpha$ )] stimulated miR-506 promoter activity in human cholangiocytes, seen by luciferase reporter assays. Experimental overexpression of miR-506 in cholangiocytes altered the cell proteomic profile (by

**Conclusions:** Overexpression of miR-506 in PBC cholangiocytes may be promoted by pro-inflammatory cytokines found upregulated in PBC livers. MiR-506 causes PBC-like features in cholangiocytes and stimulates immune activation, representing a potential therapeutic target for PBC patients.

**Disclosure of Interest:** None Declared

P05.05

## Role of the ciliopathy protein MKS1 in the homeostasis of bile duct epithelia

Yu Xiang Song<sup>1</sup>, Bouzhir Latifa<sup>1</sup>, Fatou Kouyate<sup>1</sup>, Pascale Dupuis-Williams<sup>\* 1,2</sup>

<sup>1</sup>UMR-S 1174 INSERM/Paris Sud, INSERM, Orsay, <sup>2</sup>ESPCI, Paris, France

**Corresponding author's email:** pascale.dupuis-williams@u-psud.fr

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**Introduction:** Mutations in the protein MKS1 cause severe developmental disorders such as Meckel-Gruber syndrome (MKS), characterized by cystic kidneys and liver, CNS malformation and polydactyly. In the liver, these ciliopathies are characterized by bile ducts dysgenesis with cystic and fibrotic development.

MKS1 has been shown to be localized at the transition zone and as such, involved in the ciliary membrane composition. Most analysis converge to the conclusion that in vertebrates, the depletion of MKS1 leads to impairment of Hh and Wnt signaling pathways (Breunig *et al.*, 2008; Weatherbee *et al.*, 2009; Cui *et al.*, 2011; Dowdle *et al.*, 2011; Zhao and Malicki, 2011, Whewey *et al.*, 2013).

**Results:** We reveal that MKS1 has a role in the establishment and maintenance of epithelial cell polarity, well before ciliogenesis. We show that the protein localization in the cell varies upon the state of epithelial polarity and cell spreading.

Based on MKS1 knock-down experiments, which leads to impaired cell morphology, disorganization of the epithelial monolayer and plasma membrane mechanical tension release, we propose that MKS1 modulate the interactions between actin and membranes. We further show that MKS1 is essential to catenin positioning at the adherens junctions and set-up of functional gap junctions.

We are now investigating how this new MKS1 function could influence bile duct development by analyzing the impact of MKS1 dysfunction on cell organization and polarization in 3D cultures.

**Disclosure of Interest:** None Declared

## LY3039478 a notch gamma-secretase inhibitor blocks cholangiocarcinoma growth in a patient-derived xenograft (PDX) model

Serena Mancarella\*<sup>1</sup>, Francesco Dituri<sup>1</sup>, Nada Chaoul<sup>1</sup>, Francesco Cardile<sup>2</sup>, Pasquale De Luca<sup>2</sup>, Claudio Pisano<sup>2</sup>, Gianluigi Giannelli<sup>1</sup>

<sup>1</sup>National institute of gastroenterology, Castellana Grotte (BA), <sup>2</sup>BIOGEM-MIR research institute "G. Salvatore", Ariano Irpino (AV), Italy

**Corresponding author's email:** mancarella.serena@gmail.com


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**Introduction:** Recent studies have shown that the constitutive activation of Notch signaling is associated with the development of cholangiocarcinoma (CCA). Activation of the Notch receptor follows the proteolysis by the gamma-secretase enzyme that releases the active Notch intracellular domain (NICD) in the cytosol. NICD then translocates to the nucleus and modulates the expression of several target genes that drive carcinogenesis. LY3039478 is an inhibitor of the gamma-secretase complex that induces a reduction of the NICD downstream biological effects.

**Aims:** Our goal is to investigate the effectiveness of LY3039478 against CCA tumoral progression.

**Material and Methods:** HCCT, KMCHA-1, TFK-1, MZ-CHA1 and EGI-1 CCA cell lines were treated with LY3039478 for 24 and 48h at different concentrations. Notch signaling pathway protein expression was studied by Western Blot analysis and Immunohistochemistry. A patient derived xenograft (PDX) model was established and demonstrated to match the original tissue by immunophenotypical and gene expression analysis. Mice were treated with LY3039478 at 8mg/Kg by gavage daily.

**Results:** LY3039478 inhibits Notch signaling both in vitro and in vivo. In the CCA cell lines treated, low drug concentrations (10  $\mu$ M – 0.1  $\mu$ M) decreased the levels of NICD expression. The immunostaining and microarray assay confirm the same protein expression and gene expression profiles in patient tissue and PDX tissue. Furthermore, in the PDX animals, LY3039478 significantly ( $p < 0.05$ ) reduces CCA tumoral growth compared to controls.



**Conclusions:** Here we report that inhibiting gamma-secretase activity results in a reduction of tumor progression, and that these preclinical experimental models can predict the clinical activity of LY3039478 in human CCA.

**Disclosure of Interest:** None Declared

## Sox9 is a crucial transcription factor in development of intrahepatic cholangiocarcinoma

Xiaodong Yuan<sup>\*1</sup>, Cédric Coulouarn<sup>2</sup>, Jun Li<sup>3</sup>, Steven Dooley<sup>1</sup>, Hong-Lei Weng<sup>1</sup>

<sup>1</sup>Department of Medicine II, Section Molecular Hepatology, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany, <sup>2</sup>Liver metabolisms and cancer, University of Rennes, Rennes, France, <sup>3</sup>Department of Hepatobiliary Surgery and Visceral Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany


**Corresponding author's email:** Xiaodong.Yuan@medma.uni-heidelberg.de

**Introduction:** Sex determining region Y-box9 (Sox9) controls differentiation of hepatoblasts and formation of intrahepatic bile ducts in embryonic liver. However, the effects of Sox9 in intrahepatic cholangiocarcinoma (ICC) remain largely unknown.

**Aims:** This study investigated how Sox9 impacts ICC development.

**Material and Methods:** Sox9 expression was examined in 69 ICC and 10 non-ICC patients by immunohistochemical staining. ICC gene expression signature with and without Sox9 expression was analysed with microarray analysis. The effects of Sox9 on ICC tumor features were assessed *in vitro*.

**Results:** Sox9 expression localizes to cholangiocytes and reactive ducts in non-tumor patients. Sox9 expression is strong in cancer cells from 27% of ICC patients. Sox9 positive patients presented with shorter survival time as compared to those with negative/low Sox9 scores. Furthermore, patients with high Sox9 expression died faster than those with low Sox9 expression, when receiving chemotherapy. Knockdown of Sox9 in ICC cells altered gene expression signatures associated with apoptosis, differentiation, EMT, self-renewal and stemness. Functionally, Sox9 silencing altered cell cycle and decreased proliferation, induced cell apoptosis, inhibited cell migration and increased cancer cell sensitivity to gemcitabine, which was achieved by decreasing multidrug resistance gene expression. *In vitro* experiments further demonstrated that Sox9 expression is controlled by EGFR/ERK signaling in both normal cholangiocytes and cancer cells.



**Conclusions:** Sox9 expression is associated with clinical outcome of ICC. Functionally, Sox9 contributes to development of ICC through modulation of multiple cancer cell hallmarks. Thus, Sox9 presents as potential prognostic biomarker and therapeutic target of ICC for future clinical practice.

**Disclosure of Interest:** None Declared

## Identification of AGXT as a putative target in the Mdr2<sup>-/-</sup> murine model of liver cancer

Letizia Satriano\* <sup>1</sup>, Colm J. O'Rourke<sup>1</sup>, Monika Herr<sup>2</sup>, Douglas V. Oliveira<sup>1</sup>, Beate Straub<sup>3</sup>, Arndt Vogel<sup>4</sup>, Elizabeth A. Conner<sup>5</sup>, Anna Saborowski<sup>4</sup>, Jens U. Marquardt<sup>2</sup>, Jesper B. Andersen<sup>1</sup>

<sup>1</sup>Health and Medical Science, BRIC, Copenhagen N, Denmark, <sup>2</sup>1st Department of Medicine, <sup>3</sup>Department of Pathology, University Medical Center Johannes Gutenberg University Mainz, Mainz, <sup>4</sup>Department of Gastroenterology, Hepatology and Endocrinology, Hanover Medical School, Hanover, Germany, <sup>5</sup>Center for Cancer Research, CCR Genomics Core, Genetics Branch, National Cancer Institute, NIH, Bethesda MD, United States

**Corresponding author's email:** letizia.satriano@bric.ku.dk


**Introduction:** Primary liver cancers are the second most common cause of cancer-related deaths. These malignancies are a group of diverse neoplasms, including hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA). In Western countries there is an increasing association between metabolic dysfunction and liver cancer. To investigate the early molecular changes of the disease, we combined N-nitrosodiethylamine (DEN), a carcinogenic agent, to promote the onset of both CCA and HCC progression in a *Mdr2*<sup>-/-</sup> murine model that mimics inflammatory cholangitis caused by lack of the Mdr2 P-glycoprotein in the canalicular membrane of hepatocytes.

**Aims:** To model the early onset and progression of liver cancer in the *Mdr2*<sup>-/-</sup> system, and thus, determine the genomic alterations that cause deregulated pathways in the preneoplastic onset of tumor development.

**Material and Methods:** We utilized transcriptome, DNA methylation profiling, cytokine array, liver chemistry and IHC/Tissue microarrays performed on livers obtained from FVB/n mice following conditional *Mdr2*<sup>-/-</sup> or liver DEN injury. Samples from WT/DEN, *Mdr2*<sup>-/-</sup>, *Mdr2*<sup>-/-</sup>/DEN were collected at 16, 22 or 30 weeks after DEN injection.

**Results:** HandE on liver sections revealed an acceleration in the tumor formation in the *Mdr2*<sup>-/-</sup>/DEN group. This finding was supported by the analysis of the liver function, which showed an abnormal enzymatic activity of ALT, AST and AP compared to the other groups. Furthermore, a deregulated transcriptome assessed the *Mdr2*<sup>-/-</sup>/DEN livers as the





group with the central genomic alterations. Gene Set Enrichment Analysis was carried out to assess the significant pathways that correlate with early neoplastic lesions. The xenobiotic metabolism was top scorer ( $p < 0.01$ , FDR  $< 0.25$ ). As such, a key enzyme in the liver peroxisome processing (AGXT) was shown to be downregulated compared to the normal liver (FC  $< -2.4$ ,  $p < 0.001$ ). These results were validated in four independent HCC and CCA patient cohorts, and AGXT expression displayed an inverse correlation with promoter DNA methylation. Analysis of overall survival demonstrated that low expression of AGXT is associated with worse prognosis ( $p < 0.01$ ). Although AGXT's primary function is in the glyoxylate detoxification of the liver, data suggests that AGXT also is involved in the lipid metabolism.

**Conclusions:** Our integrative molecular analysis reveal a novel role of AGXT in the preneoplastic stages of hepatic tumor growth through a deregulation of the lipid homeostasis. This emphasizes the potential for metabolic-targeted therapy in liver cancer.

**Disclosure of Interest:** None Declared

## Chronic hepatitis C is associated with increased incidence of gall bladder stones due to enhanced expression of D19H polymorphism in hepatobiliary cholesterol transporter ABCG8

**Amr S. Hanafy**\*<sup>1</sup>

*<sup>1</sup>Internal medicine department –Hepatology division, Zagazig University, Zagazig, Egypt*

**Corresponding author's email:** Dr\_amr\_hanafy@yahoo.com

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**Introduction:** ATP-binding cassette (ABC) transporters aim at regulation of lipids kinetics. The hepatobiliary excretion of cholesterol is regulated by ABCG8. D19H may represent the predominant single nucleotide polymorphism in ABCG8 with enhanced intestinal absorption of cholesterol.

**Aims:** Examine the inducing role of chronic HCV infection on the ABCG8 D19H (rs11887534) polymorphism in Egyptian patients with HCV who developed gall stones.

**Material and Methods:** A case control study included 100 chronic HCV patients with confirmed gall bladder stone disease by abdominal ultrasonography, compared to 100 patients with HCV and had no gall stones and healthy controls (n = 100). The inclusion criteria were chronic HCV without previous therapy for HCV with documented gallstones or previous cholecystectomy. Patients excluded if they had ischemic heart disease or systemic hypertension with dyslipidemia, diabetes, obesity, positive family history of dyslipidemia.

Genomic DNA was extracted and the ABCG8 gene loci was determined by using PCR-RFLP. The ABCG8 D19H was selected and amplified by PCR. Two types of alleles were evaluated; the wild D (homozygous) and H variant.

**Results:** The main age of patients with HCV and gall stones, and the 2 control groups; HCV without gall stones and healthy controls (HC)  $45.3 \pm 5.6$ ,  $43.8 \pm 8.3$ ,  $44.5 \pm 4.3$  years. 82% of the patients were males while 80% of the controls were males. The observed wild D genotype in HC was 95/100 patients, variant type (HH/HD) 5/100 patients, in HCV controls, the wild D in 80/100, variant type (HH/HD) 20/100, in HCV patients with gall stones, variant type (HH/HD) was seen in 40/100 patients, the frequency of

heterozygous H genotype was significantly higher in HCV with gall stones (40%, vs. 5%, 20%) ( $p=0.01$ ), (OR=2.56; 95% CI= 1.3 - 4.9).

Males with chronic HCV had a higher risk; 30/40 patients of variant H genotype with gall stones were males. No association between SNP of ABCG8 and lipid profile as these patients showed lower values of serum triglyceride ( $118.8 \pm 9.4$  mg/dl), total cholesterol ( $153.6 \pm 8.4$  mg/dl), and low-density lipoprotein ( $95.3 \pm 2.6$  mg/dl), interestingly HCV patients with ABCG8 D19H polymorphism showed increased carotid intima-media thickness ( $1.1 \pm 0.3$  mm) by carotid ultrasound.

**Conclusions:** The enhanced expression of ABCG8 D19H polymorphism induced by chronic HCV may be the future aim of therapeutic agents for primary prevention of gall bladder disease.

**Disclosure of Interest:** None Declared

## Yap is essential for cholangiocyte homeostasis and its removal leads to loss of ductal integrity and compensatory biliary cell expansion

**Brian J. Pepe-Mooney**<sup>\*1,2</sup>, **Michael Dill**<sup>1,2</sup>, **Wei-Chien Yuan**<sup>1,2</sup>, **Fernando Camargo**<sup>1,2</sup>

<sup>1</sup>The Stem Cell Program, Boston Children's Hospital, Boston, <sup>2</sup>Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, United States


**Corresponding author's email:** [bpepemoney@fas.harvard.edu](mailto:bpepemoney@fas.harvard.edu)

**Introduction:** Yap, the downstream activator of Hippo signaling, is an important regulator of stemness, cell proliferation, and tissue morphogenesis. In the liver, dysregulated YAP has been associated with inflammatory biliary diseases, ductular expansion, and we have previously shown that YAP drives hepatocytes to a progenitor-like cell fate. YAP is strongly expressed in biliary cells (BCs) but its function remains unknown.

**Aims:** The aim of this study was to understand the role of Yap in the regulation of BCs and its function in ductular expansion.

**Material and Methods:** We generated a reporter mouse which uses the promoter of CTGF, a Yap target gene, to drive the expression of eGFP (CTGF<sub>e</sub>GFP) to study hepatic YAP activity under normal and injury conditions. Additionally, we generated a tamoxifen (TM) inducible, biliary specific, Yap knockout (KO) mouse with a tdTomato reporter (CK19YAPKO-T), a control reporter mouse (CK19-T), and a biliary specific, diphtheria toxin receptive mouse (CK19DTA-T). Liver sections were analyzed by immunofluorescence (IF). Isolated cells were cultured as 3D organoids.

**Results:** IF analysis of CTGF<sub>e</sub>GFP showed co-localization of GFP to a subset of BCs (6.7% for A6, 10.1% for Sox9). Administration of 0.1% DDC diet to these mice led an increase in co-localization to 34.6% (41.9%, respectively), after 1 week. GFP<sup>+</sup> BCs were isolated which turned on GFP expression upon organoid culture generation, and Yap was found to be essential for organoid formation. Furthermore, analysis of CK19YAPKO-T mice compared to CK19-T mice at 3 days, 7 days, and 21 days after TM showed a loss of tomato labelling (a surrogate for Yap KO) as a percentage of total ductal cells from 33.4% to 17.7% to 12.6% respectively. Additionally, YAPCK19-T livers showed a compensatory increase in the number of pCK positive, tdTomato negative biliary-like cells around the portal areas compared to control (mean pCK<sup>+</sup> cells: 27.42 control vs. 53.2 KO). Analysis,



using a murine FLP/FRT labeling system, showed that Yap-WT biliary cells and not hepatocytes were the source for this compensatory response. This phenomenon was not observed in CK19-DTA mice, a model of acute BC death.

**Conclusions:** We found that (1) Yap activity is restricted to a subset of BC under homeostatic conditions and this number increases with injury, (2) Yap is essential for BC maintenance in vitro and in vivo, and (3) loss of Yap in BCs results in a compensatory ductular expansion from remaining Yap-WT biliary cells, that is not due to acute BC death.

**Disclosure of Interest:** None Declared

## Tridimensional cultures of human biliary tree and hepatic stem/progenitor cells in simulated microgravity perpetuated the stem features and reduced the metabolic oxidation

Daniele Costantini\*<sup>1</sup>, Vincenzo Cardinale<sup>1</sup>, Luca Casadei<sup>1</sup>, Guido Carpino<sup>2</sup>, Lorenzo Nevi<sup>1</sup>, Sabina Di Matteo<sup>1</sup>, Samira Safarikia<sup>1</sup>, Luca Verdesca<sup>1</sup>, Fabio Melandro<sup>1</sup>, Pasquale B. Berloco<sup>1</sup>, Cesare Manetti<sup>1</sup>, Eugenio Gaudio<sup>1</sup>, Domenico Alvaro<sup>1</sup>

<sup>1</sup>Sapienza university of Rome, <sup>2</sup>University of Rome "Foro Italico", Rome, Italy

**Corresponding author's email:** daniele.costantini@uniroma1.it


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**Introduction:** Gravity is fundamental for proliferation, differentiation and other cell functions.

**Aims:** The aim of the present study was to build 3D-cultures of human hepatic and biliary tree stem/progenitor cells in microgravity and study its impact on cell differentiation and metabolomics.

**Material and Methods:** Cells were cultivated in the Rotary Cell Culture System (Synthecon) to simulate weightless culture conditions. Human biliary tree stem cells (hBTSCs) primary cultures and human cells of the hepatic lineage (HepG2) were grown in microgravity or in normogravity. Self-replication and differentiation toward mature cells were determined, respectively, by culturing in Kubota's Medium (KM) and a medium tailored for hepatocyte differentiation (HDM). Gene expression by RT-qPCR and cell exo-metabolome profiles by Nuclear Magnetic Resonance (NMR) were analyzed to evaluate the effects of microgravity on cultures.

**Results:** An extensive 3D growth and an increase of stemness genes expression (OCT4, SOX17, PDX1) have shown in hBTSCs in microgravity in KM ( $p < 0.05$  vs. normogravity in KM). hBTSCs showed a defected capacity to differentiate toward mature hepatocytes in microgravity, since the administration of HDM in cultures in microgravity lowered dramatically the expression of hepatocyte lineage genes (ALB, ASBT and CYP3A4) with respect to HDM in normogravity ( $p < 0.05$ ). In HepG2, the microgravity caused a lower ( $p < 0.05$  vs. normogravity) expression of CYP3A4, a terminal differentiation gene which is expressed in hepatic acinus zone 3. Interestingly, the NMR analysis of the exo-



metabolomes showed that both cell populations in microgravity presented higher glucose consumption and lower pyruvate and glutamate consumption with respect to normogravity ( $p < 0.05$ ), associated with the release of fermentation (lactate, acetate) and ketogenesis products (B-hydroxybutyrate).

**Conclusions:** The pluripotency of human embryonic stem cells belong from their polyunsaturated metabolome. In this paper we demonstrated that, while in normogravity the differentiation of human biliary tree and hepatic stem/progenitor cells toward mature hepatocytes was associated with the oxidative phosphorylation metabolism, the microgravity maintained the stem features and attenuated the metabolic oxidation. From one side, these results should be considered in space medicine programs but, from the other side, they could be an interest tool to develop devices based on stem/progenitor cells and organ revitalization.

**Disclosure of Interest:** None Declared

## PGC-1 $\beta$ promotes development of hepatocellular carcinoma limiting ROS detrimental accumulation

Elena Piccinin<sup>1,2</sup>, Claudia Peres<sup>1,2</sup>, Elena Bellafante<sup>3</sup>, Gaetano Villani<sup>4</sup>, Antonio Moschetta<sup>1,5</sup>

<sup>1</sup>Department of Interdisciplinary Medicine, "Aldo Moro" University of Bari, Bari, <sup>2</sup>INBB, National Institute for Biostuctures and Biosystems, Rome, <sup>3</sup>Fondazione Mario Negri Sud, Santa Maria Imbaro (CH), <sup>4</sup>Department of Basic Medical Sciences, Neurosciences and Sense Organs, "Aldo Moro" University of Bari, <sup>5</sup>National Cancer Center, IRCCS Istituto Oncologico "Giovanni Paolo II", Bari, Italy

**Corresponding author's email:** antonio.moschetta@gmail.com


**Introduction:** Hepatocellular carcinoma (HCC) is one of the six most common neoplasia and the third most frequent cause of cancer death. However, the exact molecular mechanisms driving cellular neoplastic transformation are less well understood, and the therapeutic options available are limited. The peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) coactivator-1 $\beta$  (PGC-1  $\beta$ ) coactivator is the master regulator of mitochondrial biogenesis and oxidative metabolism as well as of antioxidant defense. Specifically in the liver PGC-1 $\beta$  promotes *de novo* lipogenesis, thus sustaining cellular anabolic processes.

**Aims:** Since it has been described that metabolic reprogramming is one of the key feature of cancer, and given the essential involvement of PGC-1 $\beta$  on mitochondrial biogenesis and lipid metabolism, our scientific efforts aim to unravel if PGC-1 $\beta$  can play any role in the development and progression of hepatocellular carcinoma.

**Material and Methods:** To study the role of PGC-1 $\beta$  gain and loss of function in the development of liver tumorigenesis, we generated both hepatic-specific PGC-1 $\beta$  transgenic (LivPGC-1 $\beta$ ) and PGC-1 $\beta$  knockout mice (LivPGC-1 $\beta$ KO), and we challenged them with two model of hepatic carcinogenesis (chemical and genetic).

**Results:** Our study demonstrated a pivotal role of PGC-1 $\beta$  in driving tumor development. Indeed, whereas mice overexpressing PGC-1 $\beta$  demonstrate greater tumor susceptibility, PGC-1 $\beta$  knockout mice are protected from carcinogenesis. Histological and Real time qPCR analysis revealed that high level of PGC-1 $\beta$  is able to boost ROS scavengers expression, therefore limiting the detrimental ROS accumulation and, consequently,





apoptosis. Moreover, it supports tumor metabolism through the expression of genes involved in fatty acid and tryglycerides synthesis. Accordingly, the specific hepatic ablation of PGC-1 $\beta$  promotes the accumulation of ROS-driven macromolecule damage, finally resulting in an increased cell death.

**Conclusions:** In conclusion this study illustrates that hepatic PGC-1 $\beta$  act as a gatekeeper of redox status, through orchestrating different metabolic programs to allow tumor progression. Although the role of PGC-1 $\beta$  in promoting liver injury and hepatocarcinogenesis is still not well defined, our findings support the therapeutic exploitation of oxidizing agent in the clinical management of HCC patients.

**Disclosure of Interest:** None Declared

## Characterization of the mitochondrial phenotype in primary biliary cholangitis patients' cholangiocytes

Filip Wysokinski<sup>1</sup>, Bo Meng<sup>1</sup>, Isabella Wong<sup>2</sup>, Jasper Bitner<sup>1</sup>, Claudia Zwingmann<sup>1</sup>, Shawn Wasilenko<sup>1</sup>, Weiwei Wang<sup>1</sup>, Gopinath Sutendra<sup>1</sup>, Claudia Zwingmann<sup>3</sup>, Ishwar Hosamani<sup>1</sup>, David Sharon<sup>4</sup>, Chelsea McDougall<sup>1</sup>, Evangelos Michelakis<sup>1</sup>, Andrew Mason<sup>1</sup>

<sup>1</sup>Medicine, University of Alberta, <sup>2</sup>Grant MacEwan, Edmonton, <sup>3</sup>Neuroscience Research Unit, Université de Montréal, Quebec, <sup>4</sup> Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Canada

**Corresponding author's email:** wysokins@ualberta.ca

**Introduction:** Primary Biliary Cholangitis (PBC) is a complex hepatobiliary disorder characterized by loss of bile ducts. It is an autoimmune disease because patients produce anti-mitochondrial antibodies (AMAs) and autoreactive T-cells which both target the E2 subunit of the mitochondrial pyruvate dehydrogenase complex (PDC-E2), a key regulator of glucose oxidation. AMAs also strongly react to a protein resembling PDC-E2 on the surface of bile ducts in PBC patients. However, it is unknown whether this disease specific “mitochondrial phenotype” represents excess production of PDC-E2, whether it affects cholangiocyte metabolism or how it relates to disease.

**Aims:** Assess mitochondrial and metabolic function in cholangiocytes from PBC patients and relevant controls.

**Material and Methods:** Cholangiocytes were isolated from liver transplant recipients' livers using immunomagnetic cell separation. Shotgun-proteomics was performed on cholangiocyte lysates. Confirmation of glycolytic activity was performed with isotopic glucose tracing with NMR spectroscopy and western blot for glycolytic enzyme expression. Mitochondrial respiration was measured with an oxygen biosensor plate and Seahorse XF24. Mitochondrial density was assessed by quantitative PCR (qPCR) for mitochondrial DNA (mtDNA) copy number and tetramethylrhodamine methylester (TMRM) staining which measures functional mitochondria levels.

**Results:** STRING analysis revealed enrichment in protein candidates related to glycolysis. Increased expression of the glycolytic enzyme, ENO2, was validated with western blot (p <0.05). NMR of cells and supernatants showed increases in glucose-derived lactate

in PBC BEC ( $p < 0.01$ ), indicating elevated glycolysis. The oxygen biosensor indicated mitochondrial respiration was also elevated in PBC BEC ( $p < 0.01$ ). Validation of these changes with Seahorse XF24 showed elevated glycolysis ( $p < 0.05$ ) and a trend for increased respiration in PBC BEC. QPCR showed elevated mtDNA levels in PBC BEC ( $p < 0.05$ ) indicating mitochondrial biogenesis which was supported by elevated levels of TMRM staining.

**Conclusions:** These results indicate that PBC patient's cholangiocytes have a novel metabolic phenotype with activation of catabolic pathways related to energy production. At this time it is unclear how this relates to disease pathogenesis; however, given the importance of mitochondrial function in regulating cellular viability and local inflammation, further characterization of this phenotype in cholangiocytes may provide insight into PBC pathogenesis.

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## Differential effects of FXR or TGR5 activation in cholangiocarcinoma progression

Ibone Labiano<sup>\*1,2</sup>, Oihane Erice<sup>1</sup>, Ander Arbelaiz<sup>1</sup>, Álvaro Santos-Laso<sup>1</sup>, Patricia Muñoz-Garrido<sup>1,2</sup>, Alfredo Caro-Maldonado<sup>3</sup>, Natalia Martín-Martín<sup>3</sup>, Arkaitz Carracedo<sup>3,4,5,6</sup>, Elisa Lozano<sup>2,7</sup>, Jose Juan Marín<sup>2,7</sup>, Colm J O'Rourke<sup>8</sup>, Jesper B Andersen<sup>8</sup>, Jordi Llop<sup>9</sup>, Vanessa Gómez-Vallejo<sup>9</sup>, Daniel Padro<sup>9</sup>, Abraham Martín<sup>9</sup>, Marco Marzioni<sup>10</sup>, Luciano Adorini<sup>11</sup>, Michael Trauner<sup>12</sup>, Luis Bujanda<sup>1,2</sup>, María Jesús Perugorria<sup>1,2,6</sup>, Jesús María Banales<sup>1,2,6</sup>

<sup>1</sup>Department of Liver and Gastrointestinal Diseases, Health Research Institute Biodonostia, Donostia-San Sebastian, <sup>2</sup>National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd, Instituto de Salud Carlos III, Madrid), <sup>3</sup>CIC bioGUNE, Bizkaia Technology Park, Derio, <sup>4</sup>CIBERONC, Instituto de Salud Carlos III, Madrid, <sup>5</sup>Biochemistry and Molecular Biology Department, University of the Basque Country (UPV/EHU), <sup>6</sup>IKERBASQUE, Basque Foundation for Science, Bilbao, <sup>7</sup>Experimental Hepatology and Drug Targeting (HEVEFARM), Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain, <sup>8</sup>Department of Health and Medical Sciences, Biotech Research and Innovation Centre, Copenhagen, Denmark, <sup>9</sup>Molecular Imaging Unit, CIC biomaGUNE, Donostia-San Sebastian, Spain, <sup>10</sup>Department of Gastroenterology, Università Politecnica delle Marche, Ancona, Italy, <sup>11</sup>Intercept Pharmaceuticals, New York, United States, <sup>12</sup>Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria

**Corresponding author's email:** [ibone.labiano@biodonostia.org](mailto:ibone.labiano@biodonostia.org)

**Introduction:** Cholangiocarcinomas (CCAs) are aggressive tumors with poor outcome due to late diagnosis and refractory nature. CCAs may arise under certain cholestatic conditions, where intrahepatic accumulation of bile acids may facilitate a co-carcinogenic effect by promoting cholangiocyte proliferation and inflammation, and by reducing FXR-dependent chemoprotection.

**Aims:** The differential activation of the bile acid receptors FXR or TGR5 was evaluated in CCA progression.

**Material and Methods:** FXR and TGR5 expression was determined in two different cohorts of CCA patients (i.e. Denmark and Spain), as well as in CCA cells and normal human cholangiocytes in culture. An orthotopic model of human CCA was established in immunodeficient mice and tumor growth was monitored by magnetic resonance imaging

(MRI) under chronic administration of selective FXR or TGR5 agonists, i.e. obeticholic acid (OCA) or INT-777 (Intercept Pharmaceuticals; 0,03% in chow for 2 months), respectively. The differential effects of FXR or TGR5 activation were evaluated on proliferation, apoptosis, migration and mitochondrial energetic metabolism (i.e. Seahorse Bioscience) in CCA cells *in vitro*.

**Results:** FXR is downregulated and TGR5 upregulated in human CCA tissue compared to normal surrounding liver tissue in both human cohorts. FXR correlates with tumor differentiation, whereas TGR5 correlates with perineural invasion. TGR5 expression is increased in perihilar *vs* intrahepatic CCAs. *In vitro*, FXR is downregulated and TGR5 upregulated in human CCA cells compared to normal human cholangiocytes. In mice with orthotopic implants of human CCA tumors, chronic administration of OCA inhibited the tumor growth compared to untreated control animals; this was accompanied by decreased expression of proliferation (i.e. PCNA, Ki67), biliary (i.e. CK19) and epithelial (i.e. ZO-1) markers within the tumors. In contrast, chronic administration of INT-777 *in vivo* showed no effects on CCA tumor growth. *In vitro*, OCA inhibited CCA cell proliferation and migration, associated with decreased mitochondrial energetic metabolism, and did not affect apoptosis. In contrast, INT-777 stimulated proliferation and migration of CCA cells, associated with increased mitochondrial energetic metabolism.

**Conclusions:** Activation of FXR inhibits, whereas TGR5 may promote, CCA progression through regulation of proliferation, migration and mitochondrial energetic metabolism. Regulation of FXR and/or TGR5 activities may represent potential novel therapeutic strategies for CCA.

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## Relationship of IMP3 and HER3 expression with TH17 cells in cholangiocarcinomas

Yulian Ananiev<sup>1</sup>, Koni Ivanova<sup>1</sup>, Mariana Penkova<sup>2</sup>, Maya Gulubova<sup>1</sup>

<sup>1</sup>General and clinical pathology, <sup>2</sup>Internal Medicine, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

**Corresponding author's email:** julian.r.ananiev@gmail.com

**Introduction:** Cholangiocarcinomas are uncommon but very aggressive tumors. Many molecular and cell markers plays a major role in development and progression of this disease. Different precancerosis, some tumors and normal cholangiocytes have been investigated for insulin-like growth factor II mRNA binding protein 3 (IMP3) expressions, HER2 and HER3 and correlated with disease aggressiveness and progression. In addition, T-helpers 17 (Th17) cells have been characterized as a key population anti-tumour immunity and they have major role in tumor progression.


**Aims:** The aim of our study was to investigate the expression of IMP3 and HER3, and IL-17 in three groups of patients and to explore the correlations, the clinicopathological characteristics and prognosis of cholangiocarcinoma.

**Material and Methods:** The immunohistochemical expression of IMP3, HER2 and HER3 in normal cholangiocytes, dysplastic lesions and cholangiocarcinomas was evaluated in 14 patients (5 cholangiocarcinomas, 5 cholangitis with dysplasia and 4 healthy controls). Also we investigated IL-17 expression in immune cells in these specimens.

The results were compared with clinical and pathological parameters of investigated patients.

**Results:** All specimens were negative for HER2 protein expression. The patients with cholangitis showed moderate expression of IMP3 and HER3, but this expression was stronger in cholangiocarcinomas specimens and not found in healthy controls (normal cholangiocytes) ( $\chi^2 = 5.48$ ,  $p = 0.031$ ).

The proportion of peritumoral Th17 was significantly higher in cholangiocarcinomas group than in control group ( $9.32 \pm 12.6\%$  vs.  $2.54 \pm 1.06\%$ ,  $p = 0.001$ ).



After analysis, we found that more intense expression of IMP3 in cholangiocarcinomas correlated with low number of Th17 in peritumoral area ( $\chi^2 = 10.2$ ,  $p = 0.04$ ).

**Conclusions:** Our results suggest that IMP3 and HER3 production by tumor cells may affect the tumor environment via suppression of Th17 and it could be a useful predictor of prognosis of cholangiocarcinomas.

**Disclosure of Interest:** None Declared

## Risk reduction with obeticholic acid treatment in patients with primary biliary cholangitis not achieving the POISE primary endpoint

**Maren H. Harms<sup>1</sup>, Marco Carbone<sup>2, 3</sup>, Bettina E. Hansen<sup>1, 4</sup>, George Mells<sup>2</sup>, Richard Pencek<sup>5</sup>, Elizabeth Malecha<sup>5</sup>, Leigh MacConell<sup>5</sup>**

*<sup>1</sup>Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands, <sup>2</sup>Academic Department of Medical Genetics, University of Cambridge, Cambridge, United Kingdom, <sup>3</sup>Center for Autoimmune Liver Disease, Humanitas Clinical and Research Institute, Milan, Italy, <sup>4</sup>Institute of Health Policy, Management and Evaluation, University of Toronto, Toronto, Canada, <sup>5</sup>Intercept Pharmaceuticals, Inc., San Diego, United States*

**Corresponding author's email:** [m.h.harms@erasmusmc.nl](mailto:m.h.harms@erasmusmc.nl)

**Introduction:** The Global PBC study group and the UK-PBC consortium have developed and validated continuous prognostic models, based on data from >6000 UDCA-treated patients with primary biliary cholangitis (PBC), which estimate the risk of an endpoint (liver transplantation [LT] and all-cause mortality or liver-related mortality, respectively) in patients with PBC. POISE was a double-blind, 12-month, placebo-controlled, phase 3 trial assessing the efficacy of obeticholic acid (OCA) 5 and 10 mg daily in patients with PBC. POISE utilized dichotomous response criteria (ALP <1.67x ULN with total bilirubin  $\leq$ ULN and  $\geq$ 15% reduction in ALP) to assess the efficacy of OCA treatment.

**Aims:** The objective of this analysis was to assess the risk reduction of patients in POISE who did not achieve the primary endpoint, using the GLOBE score and UK-PBC risk score.

**Material and Methods:** Patients who were intolerant or had an inadequate response to UDCA were randomized and dosed with Placebo, OCA 5-10 mg, and OCA 10 mg. Baseline and Month 12 data for patients not achieving the POISE primary endpoint (Placebo, n=66; OCA 5-10 mg, n=38; OCA 10 mg, n=39) were entered into the UK-PBC and GLOBE score calculations, to assess the risk of an endpoint in 5, 10, and 15 years.

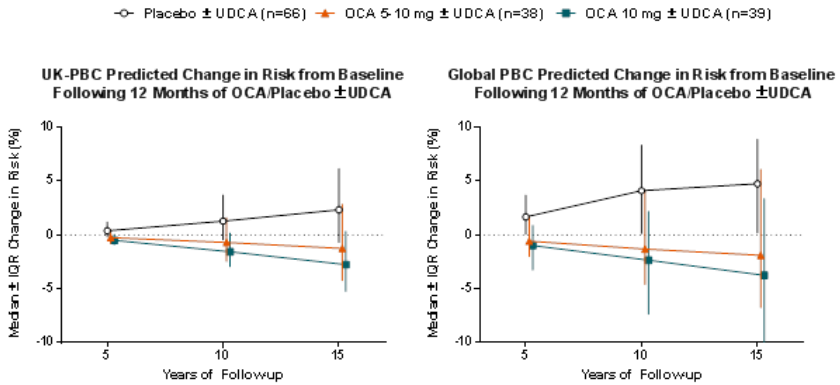
**Results:** At baseline, 91% of patients were receiving UDCA (mean [SD] dose: 16 [4] mg/kg). Despite receiving the standard of care treatment for PBC, the estimated risk of LT or mortality increased at all time points for patients in the placebo group (**Figure 1**). In



contrast, 12 months of OCA treatment resulted in significant reductions in estimated risk at 5, 10, and 15 years in both OCA treatment groups, with either risk score ( $p < 0.01$ ). After 12 months of treatment, the median (Q1, Q3) reduction in ALP (U/L) was -65.1 (-171.9, -14.6) and -120.3 (-152.1, -61.6) in the OCA 5-10 mg and OCA 10 mg groups, respectively, compared to -8.5 (-55.3, 32.1) in the Placebo group.

**Conclusions:** Although patients in this analysis did not achieve the dichotomous POISE primary endpoint, OCA treatment resulted in a significant reduction in estimated risk of LT and (all-cause vs liver-related) mortality with both the GLOBE score and UK-PBC risk score.

**Figure:**



$p < 0.01$  for both OCA treatment groups for UK-PBC and Global PBC prognostic models. P-value for comparing active treatments to Placebo is obtained using the Wilcoxon rank-sum test.

**Disclosure of Interest:** M. Harms, M. Carbone, B. Hansen, G. Mells: : None Declared, R. Pencek, E. Malecha, L. MacConell: Employee: Intercept Pharmaceuticals, Inc.

## 3D-reconstruction of the murine biliary tree during cholestasis using two different techniques: corrosion casts and Microfil®-MV. Methods` descriptions and first results

**Beate Richter<sup>1</sup>**, **S Zafarnia<sup>2</sup>**, **F. Gremse<sup>2</sup>**, **F. Kiessling<sup>2</sup>**, **U. Settmacher<sup>3</sup>**, **U. Dahmen<sup>1</sup>** and **Experimental Surgery, Department of General, Visceral and Vascular Surgery, University Hospital Jena, Jena, Germany**

<sup>1</sup>Experimental Surgery, University of Jena, Jena, <sup>2</sup>Experimental molecular imaging, University of Aachen, Aachen, <sup>3</sup>General, Visceral and Vascular Surgery, University Hospital, Jena, Germany

**Corresponding author's email:** Beate.Richter@med.uni-jena.de


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**Introduction:** Obstructive cholestasis can lead to significant alterations of the hepatic architecture. Current studies reported about advantages in techniques for 3D-Reconstruction of delicate vascular structures in small animals.

**Aims:** We wanted to test the hypothesis that established techniques offer a fast and reliable tool for detection of time-depending changes in the three-dimensional (3D) architecture of the murine biliary tree (BT) during cholestasis. We used two different techniques: radiopaque Microfil®-MV (MV) for 3D-reconstruction (3D-Reco) using a micro-CT ( $\mu$ CT) and Corrosion Cast (CC) for focused imaging using digital-stereo microscope.

**Material and Methods:** We induced cholestasis by ligation of the main extrahepatic bile duct (BDL) in 20 male mice (C57BL/6N, 20-25 g). We injected the media (each n=10) into the main extrahepatic bile duct at several postoperative days after BDL (POD:1,3,5,7,14,28). MV as a silicone based injection compound (FlowTech.Inc.,USA) cured to a soft radiopaque 3D-cast. The whole liver was scanned in a  $\mu$ CT enabling a 3D-Reconstruction (using "IMALYTICS Preclinical", kindly provided by Prof. Kießling) of the contrasted BT. CC (Batson No.17, Polysciences, USA) as a multicomponent polymer with an added coloured pigment cured to a solid 3D-cast allowing focused imaging using a digital stereo microscope (Leica M60+IC80HD).

**Results:** Due to inconsistently increased intraductal pressure levels of the simultaneously vulnerable BT we obtained utilizable samples only using the hand-pressure injection technique. In 65% of the animals (13/20) we achieved to generate samples enabling evaluation either using  $\mu$ CT-Scan (MV, n=5) or focused imaging (Corrosion Cast, n=7).



In 2 animals (MV: n=2) an incurable leakage of the extrahepatic bile duct prevented filling of the BT. In 6 samples we found an intrahepatic leakage (MV: n=1 and CC: n=2) or incomplete filling of the intrahepatic bile ducts (MV: n=2 and CC: n=1). All samples showed dilatation of the intrahepatic bile ducts of the 2nd and 3rd generation (BD) 24hrs after BDL. MV allowed a fast 3D-Reco of the BT. In CC we determined a reticular pattern of dilated BD til POD 5, followed by an increasing number of cotton ball like shaped BD until POD 14 and at POD 28 we found a highly dense mesh of BD.

**Conclusions:** Both techniques harbor a challenge with filling the complete BT in mice. Once established, MV and CC provide reliable and fast 3D-Reco tools to address focused evaluation of kinetic alterations of the BT.

**Disclosure of Interest:** None Declared

## Colesevelam attenuates cholestatic liver and bile duct injury in Mdr2<sup>-/-</sup> mice by modulating composition, signaling and excretion of fecal bile acids

Claudia D. Fuchs<sup>1</sup>, Gustav Paumgartner<sup>1</sup>, Emina Halilbasic<sup>1</sup>, Annika Wahlström<sup>2</sup>, Nadja Leditznig<sup>1</sup>, Andrea Thüringer<sup>3</sup>, Marcus Stahlman<sup>2</sup>, Tatjana Stojakovic<sup>4</sup>, Karl Kashofer<sup>3</sup>, Hanns-Ulrich Marschall<sup>2</sup>, Michael Trauner<sup>1</sup>

<sup>1</sup>Gastroenterology and Hepatology, Medical University of Vienna, Vienna, Austria, <sup>2</sup>Molecular and Clinical Medicine, University of Gothenburg, Gothenburg, Sweden, <sup>3</sup>Institute of Pathology, <sup>4</sup>Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria


**Corresponding author's email:** claudia.fuchs@meduniwien.ac.at

**Introduction:** Following their biliary excretion, bile acids (BAs) are reabsorbed from the intestine and recirculate back to the liver. Interruption of the enterohepatic circulation of BAs may protect against BA-mediated cholestatic liver and bile duct injury. BA sequestrants are used for symptomatic treatment of cholestatic pruritus but their impact on the underlying cholestasis is still unclear.

**Aims:** We aimed to explore the therapeutic effects of the BA sequestrant Colesevelam (CS) and potential mechanistic differences to selective uptake (ASBT) inhibition in improving cholestasis, inflammation and biliary fibrosis.

**Material and Methods:** Mdr2<sup>-/-</sup> mice (as mouse model of cholestasis and sclerosing cholangitis) received CS (2% w/w diet) for 8 weeks. Gene expression profiles in liver and intestine were assessed via RT-PCR. GLP-1 levels in portal blood were measured by ELISA and immunohistochemistry was performed from colon.  $\alpha$ SMA and OH-proline were assessed at protein levels. 16s rRNA microbiota analysis as also performed.

**Results:** CS improved serum liver enzymes (AST, ALT, AP) and BA levels, hepatic expression of pro-inflammatory (*Tnf- $\alpha$*  by -90%; *Vcam1* -95%; *Mcp-1* -90%) and pro-fibrogenic (*Col1a1* by -90%; *Col1a2* -90%) genes as well as bile duct proliferation (*CK19* -95%). At protein levels CS reduced OH-proline and  $\alpha$ SMA ( $p \leq 0.001$ ). Total fecal BA output increased 6-fold by CS treatment consisting of more than 50% of secondary BAs such as (tauro)LCA and (tauro)DCA (TGR5 ligands). Serum GLP-1 levels and mRNA expression of *Proglucagon* were increased in CS-fed mice 3.5-fold and 5-fold, respectively,



but not by ASBT inhibition. Of note, *Fgf15* levels were completely abolished by CS. Microbiota analysis revealed an increase of d-proteobacteria in CS-fed *Mdr2*<sup>-/-</sup> mice as well as a shift from clostridiales to lactobacillus.

**Conclusions:** In addition to ameliorating bile toxicity by promoting fecal BA elimination, CS favors metabolism of primary into secondary BAs, resulting in TGR5-mediated secretion of potentially cholangioprotective GLP-1 from L-cells (not seen after ASBT inhibition), thus effectively improving cholestatic liver and bile duct injury (sclerosing cholangitis) in *Mdr2*<sup>-/-</sup> mice.

**Disclosure of Interest:** None Declared

## S-Adenosyl L-Methionine-induced protein S-glutathionylation may modulate immune responses in patients with primary biliary cholangitis

Ewa Kilanczyk<sup>1</sup>, Ewa Wunsch<sup>2</sup>, Piotr Milkiewicz<sup>2,3</sup>, Malgorzata Milkiewicz<sup>1</sup>

<sup>1</sup>Department of Medical Biology, MCD1, <sup>2</sup>Translational Medicine Group, Pomeranian Medical University, Szczecin, <sup>3</sup>Liver and Internal Medicine Unit, Medical University of Warsaw, Warsaw, Poland

**Corresponding author's email:** ewakilanczyk@gmail.com

**Introduction:** S-Adenosyl L-Methionine (SAME) as a precursor of glutathione plays an role in amelioration of processes related to oxidative stress (OS). SAME synthesis is impaired in Primary Biliary Cholangitis (PBC). Manganese superoxide dismutase (MnSOD) is a mitochondrial antioxidant enzyme that detoxifies reactive oxygen species. The production of antimitochondrial antibodies (AMAs), which are pathognomonic for PBC was linked to S-glutathionylation<sup>1</sup>.

**Aims:** Was to investigate the effect of SAME on OS parameters and to determine whether SAME alters protein S-glutathionylation and in turn affects the titre of mitochondrial autoantibodies in PBC.

**Material and Methods:** Seventeen females (mean age  $54 \pm 7.9$  years) with PBC were treated with SAME (1200 mg/bd) for 6 months. Serum samples were collected at 5 time points (0, 2 weeks, 1, 3 and 6 months). MnSOD, protein S-glutathionylation, AMA titre, total antioxidant activity (TAC) and lipid peroxidation (MDA) were evaluated in serum by commercially available ELISA assays.

**Results:** Nine patients responded to SAME supplementation with a significant increase in serum MnSOD levels at 2 weeks ( $p=0.0002$ ). Observed higher level of MnSOD was associated with the increased serum protein S-glutathionylation at 2 weeks ( $p=0.008$ ), which remained elevated after 3 and 6 mo. ( $p=0.001$ ,  $p=0.03$ , respectively). Furthermore, this protein modification was associated with the reduction of serum AMA titres (20 % reduction at 2 weeks and 1 mo. followed by 35% reduction at 3mo. ( $p=0.007$ ,  $p=0.02$  and  $p=0.002$  vs. the values evaluated at point 0, respectively). In the second group of patients ( $n=8$ ), SAME supplementation reduced MnSOD levels by 20 % after 1,3, and 6

months of treatment ( $p=0.01$ ,  $p=0.01$  and  $p=0.03$ , respectively). These changes did not affect serum protein S-glutathionylation and AMA titres.

Patients in whom SAME increased MnSOD were younger at the diagnosis (44.1 vs. 52.7 years,  $p=0.03$ ), had a shorter duration of the disease (4.1 vs. 9.0 years,  $p=0.07$ ) and a lower level of serum S-glutathionylated proteins at point 0 ( $p=0.002$ ). There was also a trend towards higher ALP activity in this group (234 vs. 175 IU/l  $p=0.07$ ). SAME treatment did not affect serum levels of TAC and MDA.

**Conclusions:** Our results suggest that SAME *via* its involvement in antioxidant response and S-glutathionylation may modulate immune responses in a proportion of patients with PBC. These findings may provide a new insight into the molecular event leading to the development of this condition.

<sup>1</sup>Hu B *et. al* (2012)

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**Disclosure of Interest:** None Declared

## Precision-cut bile duct slices as a model to study regeneration of bile ducts of human donor livers after ischemic preservation injury

Iris De Jong<sup>\*1</sup>, Alix Matton<sup>1</sup>, Su Suriguga<sup>2</sup>, Jasper van Praagh<sup>2</sup>, Wouter van Haaften<sup>2</sup>, Janneke Wiersema-Buist<sup>3</sup>, Raditya Iswandana<sup>2</sup>, Dorenda Oosterhuis<sup>2</sup>, Anette Gouw<sup>4</sup>, Ton Lisman<sup>3</sup>, Peter Olinga<sup>2</sup>, Robert Porte<sup>1</sup> and Hepatobiliary surgery and liver transplantation

<sup>1</sup>Hepato-pancreato biliary surgery, <sup>2</sup>Pharmaceutical Technology and Biopharmacy, <sup>3</sup>Surgical Research Laboratory, <sup>4</sup>Department of Pathology, University Medical Center Groningen, Groningen, The Netherlands

**Corresponding author's email:** i.e.m.de.jong@umcg.nl


**Introduction:** Clinical studies have shown a high incidence of biliary epithelial injury after cold ischemic preservation of donor livers. Insufficient epithelial regeneration from the peribiliary glands (PBGs) has been proposed as a critical mechanism in the pathogenesis of biliary strictures after transplantation. Severe biliary epithelial injury requires proliferation and mobilization of biliary progenitor cells nested in PBGs. Studies on the pathogenesis and prevention of biliary strictures are hampered by the lack of suitable laboratory and animal models. Precision-cut tissue slices are a widely used *ex vivo* technique, in which thin slices of human tissue are cultured and kept viable with intact intercellular and cell-matrix interactions for up to several days.

**Aims:** The aim of this study was to generate precision-cut bile duct slices and study their suitability as a model to study the regenerative capacity of PBGs in donor bile ducts.

**Material and Methods:** Large bile ducts of cold preserved livers declined for transplantation (n=10) were isolated and sliced, using a Krumdieck slicer. Bile duct slices were cultured in Williams' medium E up to 144 hr. Histology and immunohistochemistry (HE, Ki-67, and K19) were performed to assess cell morphology.

**Results:** Viable K19-positive PBG cells were observed at all time points. Cell proliferation rate, as assessed by Ki-67 staining, increased from 0% at 24 hr to 25% at 96 hr of culture. Proliferation rate was significantly higher in bile ducts of donors <60 years, compared to donors ≥60 years (21% vs. 11%, p=0.026)





**Conclusions:** Precision-cut bile duct slices are a feasible model to study biliary epithelial regeneration of ischemic injured donor liver bile ducts. This model provides a new *ex vivo* tool to develop pharmacological strategies to prevent post-transplant biliary strictures.

**Disclosure of Interest:** None Declared

## Autoimmune hepatitis: Evaluation of specificity of various histological features and development of a modified scoring system

Joanna Gibson<sup>\*1</sup>, Ananta Gurung<sup>2</sup>, David Assis<sup>3</sup>, James Boyer<sup>3</sup>, Dhanpat Jain<sup>1</sup>

<sup>1</sup>Department of Pathology, Yale University School of Medicine, New Haven, United States,

<sup>2</sup>Department of Pathology, University of British Columbia, Vancouver, Canada, <sup>3</sup>Internal medicine, Digestive Disease Division, Yale University School of Medicine, New Haven, United States


**Corresponding author's email:** joanna.gibson@yale.edu

**Introduction:** The current histological criteria for autoimmune hepatitis (AIH) rely heavily on lymphoplasmacytic infiltrates in portal tracts, emperipolesis and rosette formation. Recent data suggest that these features are not specific.

**Aims:** Our aim is to assess AIH histologic features and develop a new scoring system.

**Material and Methods:** We retrospectively identified 43 patients with pre-treatment liver biopsies and confirmed AIH based on clinicopathological features. Biopsies (matched for grade and stage) from 42 Hepatitis C (HCV) patients served as controls. Histological features evaluated were: inflammatory infiltrate, emperipolesis, rosettes, Kupffer cell hyaline globules (KcHG), Russell bodies, portal and lobular plasma cell (PC) clusters (defined as  $\geq 5$  PC), cholestasis, bile duct injury and endothelialitis. The inflammatory infiltrate was classified as plasma-lymphocytic (PLI) ( $>50\%$  PC) or lympho-plasmacytic (LPI) ( $\leq 50\%$  PC).

**Results:** PLI was more frequent in AIH ( $n=13$ , 30%,  $p=0.003$ ) compared to HCV controls ( $n=2$ , 5%), while LPI was more frequent in HCV ( $n=40$ , 95%) biopsies than in AIH ( $n=27$ , 63%,  $p=0.0003$ ). Portal and lobular PC clusters were significantly more frequent in AIH vs. HCV (60% vs. 26%,  $p=0.002$ ; 35% vs. 5%,  $p=0.001$ , respectively). Rosettes and emperipolesis, while more frequent in AIH vs. HCV (37% vs. 17%,  $p < 0.05$ , 51% vs. 24%,  $p=0.01$ , respectively), did not show significant association with AIH when controlled for inflammatory grade (16% vs 15%,  $p=1$ , 32% vs 23%,  $p=0.4$ , respectively). KcHG were seen in 24% of HCV biopsies vs 50% of AIH biopsies ( $p=0.03$ ). Using current AIH scoring system, all AIH and HCV biopsies in this study meet histological AIH criteria ('typical' or 'compatible'), suggesting high sensitivity and poor specificity. We propose a new scoring system: 'typical' AIH features require prominent PC (PC  $\geq 20\%$  OR



PC clusters) AND KcHG; ‘compatible’ features include prominent PC without KcHG; and ‘atypical’ features include the presence of another disease. Using these criteria, 77% AIH cases are ‘typical’ or ‘compatible’ with AIH (versus 34% HCV) and 23% are ‘atypical’ (versus 67% of controls). Application of these criteria decreases sensitivity to 77% (vs. 100%), but increases specificity to 67% (vs. 0%) compared to current criteria.

**Conclusions:** The current AIH scoring system overemphasizes the significance of emperipolesis and rosettes. We propose a new scoring system with higher specificity based on a *plasmalymphocytic* inflammation, Kupffer cell hyaline globules and plasma cell clusters.

**Disclosure of Interest:** None Declared

## Splanchnic release contributes to the elevated pool of FGF19 in the circulation of patients with obstructive cholestasis

**Kiran V. Koelfat**<sup>\*1,2</sup>, **Evelien P. Neis**<sup>1,2</sup>, **Sander Rensen**<sup>1,2</sup>, **Peter L. Jansen**<sup>1</sup>, **Cornelis H. Dejong**<sup>1,2</sup>, **Frank G. Schaap**<sup>1,2</sup>, **Steven W. Olde Damink**<sup>1,2,3</sup>

<sup>1</sup>*Surgery, Maastricht University*, <sup>2</sup>*NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht, The Netherlands*, <sup>3</sup>*Institute for Liver and Digestive Health, Royal Free Hospitals, University College London, London, United Kingdom*


**Corresponding author's email:** k.koelfat@maastrichtuniversity.nl

**Introduction:** An interrupted enterohepatic circulation disrupts feedback regulation on hepatic bile salt synthesis. Patients with obstructive cholestasis (OC) have elevated serum levels of FGF19, an FXR-regulated ileal enterokine that represses bile salt synthesis. We postulate that the liver is the source of FGF19 during OC to provide adaptation against cholestatic injury.

**Aims:** The aim was to calculate venous-arterial differences ( $\Delta$ VA) of FGF19 across major abdominal organs in patients with OC.

**Material and Methods:** Perioperative levels of bile salts (BS), FGF19 and C4 (marker for bile salt synthesis) were determined in arterial blood (RA), portal (PV), hepatic (HV), superior (SMV) and inferior mesenteric (IMV) veins of patients with (n=6) and without (n=14) cholestasis, who underwent pancreatic surgery for various indications. We calculated  $\Delta$ VA across the liver, small and large intestine to study the source of FGF19 in OC. Comparisons between the two groups were made using the Mann Whitney U Test.

**Results:** Patients with OC had higher levels of bilirubin, AP, GGT, ALT and AST, confirming cholestasis and hepatobiliary injury. FGF19 levels were higher in all vessels of patients with OC (P values <0.05). Apart from the SMV, BS levels were also increased in all vessels in the OC group (P values <0.05). In controls, both FGF19 and BS levels were higher in the SMV than in the IMV (P values <0.001). These differences were not observed in the OC group. There was no significant release of FGF19 by the small or large intestine, nor significant uptake by the liver in either group, implicating a balance of FGF19 across the gut-liver axis. Nonetheless, we observed higher splanchnic (HV-A) release of FGF19 (P=0.03) in patients with OC, suggesting increased production across the splanchnic area



in OC. In controls, BS were released by the small intestine ( $P < 0.001$ ), but not by the large intestine ( $P = 0.24$ ), and taken up by the liver ( $P < 0.001$ ). VA-differences of BS across the small intestine and liver in patients with OC were not observed. However, a trend was noted ( $P = 0.09$ ) towards increased release of BS by the large intestine in the OC group. C4 levels were decreased in OC-patients in arterial, portal and hepatic venous blood, indicating strong down-regulation of the bile salt synthetic enzyme *CYP7A1* ( $P < 0.05$ ).

**Conclusions:** This study reveals that circulating levels of FGF19 across the gut-liver axis are elevated during OC. FGF19 is released by the splanchnic area under this condition, but production by the liver could not explain this.

**Disclosure of Interest:** None Declared

## Hypothermic oxygenated machine perfusion reduces biliary reperfusion injury after transplantation of donation after circulatory death livers

Rianne Van Rijn<sup>\* 1</sup>, Otto B. van Leeuwen<sup>1</sup>, Alix P. Matton<sup>1</sup>, Laura C. Burlage<sup>1</sup>, Marius C. van den Heuvel<sup>2</sup>, Ruben H. de Kleine<sup>1</sup>, Marieke T. de Boer<sup>1</sup>, Annette S. Gouw<sup>2</sup>, Robert J. Porte<sup>1</sup>

<sup>1</sup>Surgery, <sup>2</sup>Pathology, University Medical Center Groningen, Groningen, The Netherlands

Corresponding author's email: r.van.rijn@umcg.nl

**Introduction:** Donation after circulatory death (DCD) livers are associated with an increased risk of biliary complications due to ischemia-reperfusion (IR) injury. Dual hypothermic oxygenated machine perfusion (DHOPE) of the liver has been advocated as a method to reduce IR injury.

**Aims:** The aim of this study was to determine whether DHOPE reduces IR injury of the bile ducts in DCD liver transplantation.

**Material and Methods:** In a recently performed phase 1 trial, ten DCD livers were preserved with DHOPE after static cold storage (SCS) (www.trialregister.nl NTR4493). Bile duct biopsies were obtained at the end of SCS (before DHOPE; baseline) and after graft reperfusion in the recipient. Histological severity of biliary injury was graded according to an established semi-quantitative grading system in a blinded fashion. Twenty liver transplantations using a DCD liver graft that was not preserved with DHOPE served as controls.

**Results:** Baseline characteristics were comparable between the two groups. As expected, the degree of bile duct injury at baseline (end of SCS) was similar between the two groups. After reperfusion, in the control group, the degree of biliary stroma necrosis ( $p=0.004$ ) and injury of the periluminal peribiliary glands ( $p=0.017$ ) increased, compared to baseline. In contrast, in DHOPE preserved livers the degree of bile duct injury after reperfusion did not increase compared to baseline. Moreover, there was less injury of periluminal ( $p=0.043$ ) and deep peribiliary glands ( $p=0.043$ ) after graft reperfusion in the DHOPE group, compared to controls.



**Conclusions:** This study suggests that DHOPE reduces IR injury of bile ducts after DCD liver transplantation. Whether DHOPE leads to a lower incidence of biliary complications after DCD liver transplantation, is currently investigated in a multicenter randomized controlled trial.

**Disclosure of Interest:** None Declared

## Functional role of extracellular signal-regulated kinase 5 on cholangiocarcinoma cells

Alessandra Gentilini\* <sup>1</sup>, Alessandra Caligiuri<sup>1</sup>, Giovanni Di Maira<sup>1</sup>, Mirella Pastore<sup>1</sup>, Elisabetta Rovida<sup>2</sup>, Fabio Marra<sup>1</sup>

<sup>1</sup>Università di Firenze, Dipartimento di medicina sperimentale e clinica, <sup>2</sup>Dipartimento di Scienze Biomediche, Sperimentali e Cliniche Mario Serio, Università di Firenze, Florence, Italy

**Corresponding author's email:** [alessandra.gentilini@unifi.it](mailto:alessandra.gentilini@unifi.it)

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**Introduction:** Cholangiocarcinoma (CCA) is the second most common primary hepatobiliary malignancy in the United States. Migration, invasion, growth and survival of cholangiocarcinoma cells are sustained by soluble mediators secreted by stromal cells of the surrounding connective tissue. Among these, epidermal growth factor (EGF) has been shown to contribute to CCA development, and overexpression of the EGFR has been associated with tumour progression. Signaling pathways downstream of EGFR include different members of the mitogen-activated protein kinase family, among which emerges the extracellular signal-regulated kinase 5 (ERK5). A close involvement of ERK5 pathway in the pathogenesis of cancer is demonstrated by the observation that ERK5 is upregulated in highly aggressive forms of breast and prostate cancer and in primary human hepatocarcinoma (HCC). Additionally, ERK5 is implicated in cytoskeletal remodeling and cell motility.

**Aims:** The purpose of the present study was to investigate the expression and the role of ERK5 in CCA cells.

**Material and Methods:** HuCCT-1, an intrahepatic cholangiocarcinoma cell line, was used in this study. Cell motility and invasion were assessed using modified Boyden chambers. Cell growth was determined by cell counting and BrdU incorporation assay. Cell survival was determined by MTT. P-ERK5, ERK5, p27 and cleaved PARP protein expressions were investigated by Western blotting. Cell cycle analysis was conducted by incorporation of propidium iodide using a FACSCanto flow cytometer.

**Results:** Both ERK5 and its phosphorylated form were expressed by HuCCT-1 cells at the protein level, and p-ERK5 was upregulated by cell exposure to EGF. Two specific pharmacologic inhibitors, XMD8-92 and AX 15836 were used to inhibit ERK5 activity. EGF-induced survival, migration and invasion were reduced by XMD8-92, at the doses of





5  $\mu$ M and 10  $\mu$ M. Similar effects were obtained when cells were stimulated with complete medium. Interestingly, XMD8-92 increased migration and invasion in medium deprived of serum. Moreover, cell cycle analysis showed more prolonged phases G<sub>0</sub>/G<sub>1</sub> in cells treated with XMD8-92 compared to control and P27 and cleaved PARP expressions resulted increased by this inhibitor. AX 15836, a more specific inhibitor of ERK5 activity confirmed an important role of this protein in EGF- and FBS-induced chemotaxis and invasion.

**Conclusions:** In HuCCT-1 cells, ERK5 activity plays an important role in mediating HuCCT-1 growth, survival and motility.

**Disclosure of Interest:** None Declared

## Identification of tumour suppressive and oncogenic microRNAs in gallbladder carcinoma

**Benjamin Goeppert<sup>\*1</sup>, Marcus Renner<sup>1</sup>, Felicia Truckenmueller<sup>1</sup>, Angelika Fraas<sup>1</sup>, Carsten Sticht<sup>2</sup>, Norbert Greetz<sup>2</sup>, Dominique Scherer<sup>3</sup>, Justo Lorenzo Bermejo<sup>3</sup>, Peter Schirmacher<sup>1</sup>, Stephanie Roessler<sup>1</sup>**

*<sup>1</sup>University Hospital Heidelberg, Heidelberg, <sup>2</sup>Center of Medical Research, University Hospital Mannheim, Mannheim, <sup>3</sup>ute of Medical Biometry and Informatics, University of Heidelberg, Heidelberg, Germany*


**Corresponding author's email:** benjamin.goeppert@med.uni-heidelberg.de

**Introduction:** Gallbladder carcinoma (GBC) is a rare and understudied cancer entity. GBC treatment options are very limited and 2-year survival rates of unresectable GBC is less than 10%. Radical surgery is the only potentially curative treatment option but due to late diagnosis few patients are eligible. Therefore, the development of new treatment options, including targeted therapy for GBC is required to improve patient outcome.

**Aims:** To identify tumor suppressive and oncogenic miRNAs in gallbladder carcinoma.

**Material and Methods:** We performed global miRNA profiling of 40 GBC and 8 normal gallbladder tissues. MiRNAs that are associated with survival were identified and candidate miRNAs were functionally analyzed in cholangiocellular carcinoma cell lines. Cell proliferation and colony formation assays were performed to using miRNA mimic expression in cell lines with low endogenous levels. In addition, we performed Affymetrix gene expression microarray analysis of cell lines transfected with miRNA mimics or control.

**Results:** In the miRNA profiles of 40 GBC and 8 normal gallbladder tissues, we found 992 out of 2006 miRNAs to be differentially expressed (FDR <0.001). In addition, the GBC cohort showed high heterogeneity and survival-related subgroups were identified. To select key survival associated miRNA genes, we split our cohort of 40 GBC into two groups based on median survival (18 months). This revealed 8 miRNAs to be down and 16 to be up regulated in the poor outcome group (p <0.05). The most down regulated miRNA was miR-145-5p and the top up regulated miRNA was miR-575. Overexpression of miR-145 led to a significant reduction of cell proliferation and colony formation. In contrary, ectopic expression of miR-575 resulted in an increased proliferation rate and



colony formation capacity. Gene expression profiling of cell lines expressing miR-145 mimic revealed activation of the STAT1 signaling pathway in cholangiocellular but not hepatocellular carcinoma cell lines. In addition, ERBB2 and ERBB3 which have been shown to be mutated in GBC were down regulated upon miR-145 expression. Thus, loss of miR-145 expression in GBC patients with poor outcome may lead to increased ERBB expression.

**Conclusions:** MiRNA profiling of a clinicopathological well-characterized German GBC cohort identified pro- and anti-tumorigenic miRNAs. Functional validation confirmed the tumor suppressive function of miR-145. In addition, miR145 was found to activate STAT1 and to repress ERBB signaling.

**Disclosure of Interest:** None Declared

## Biliary microrhamartomas (Von-Meyenburg complexes) are frequently associated with intrahepatic and hilar cholangiocarcinomas in liver

Joanna Gibson<sup>\*1</sup>, Lihui Qin<sup>2</sup>, Vikram Deshpande<sup>3</sup>, Dhanpat Jain<sup>1</sup>

<sup>1</sup>Department of Pathology, Yale University School of Medicine, New Haven, <sup>2</sup>Department of Pathology, Mount Sinai Medical Center, New York, <sup>3</sup>Department of Pathology, Massachusetts General Hospital, Boston, United States

Corresponding author's email: joanna.gibson@yale.edu

**Introduction:** More than 80% of intrahepatic cholangiocarcinomas (CC) arise in non-cirrhotic livers, often without any underlying chronic liver disease. The precursor lesions for sporadic CC are unknown. We have previously shown morphologic and molecular evidence that biliary-microhamartomas /Von-Meyenburg complexes (VMC) can occasionally transform into pre-neoplastic adenomatous lesions and I-CC. VMCs are known to increase with age and we suspect majority of sporadic CC arise from VMCs.

**Material and Methods:** All hepatic resections or explants containing CC from three institutions during 1985-2008 period were studied. Only intrahepatic (I-CC) and hilar (H-CC) tumors were included in the study. Numbers of VMCs present within and outside the tumors were recorded, and presence of any intermediate lesions/adenomatous transformation was specifically looked for. The tumors were histologically classified as per WHO classification. In addition, tumors that showed architectural resemblance to VMC, characterized by angular profiles of the tumor glands, dilated lumen often containing inspissated secretions/bile and with intervening hyalinized/desmoplastic stroma, were called "VMC-like". Age and sex matched hepatic resection for metastatic colonic carcinoma were used as controls.

**Results:** A total of 83 cases of CC (53 I-CC, 30 H-CC) were identified. Of these 50 (60%) had no known background liver disease and 15 (18%) had cirrhosis. Associated liver disorders included sclerosing cholangitis (primary/secondary) (14), chronic viral hepatitis B or C (7), non-alcoholic steatohepatitis (6) and others miscellaneous disorders (6). VMCs were identified in 17 (32%) of I-CC and 7 (23%) of H-CC. VMCs were present within the tumor (9 cases), outside the tumor (6 cases), or in both locations (9 cases).

Two or more VMCs were seen in 14 cases. VMCs with dysplasia/carcinoma in-situ were seen in 2 cases, and intermediate forms/adenomatous transformation was present in 6 cases. Only 7% of controls showed presence of VMC compared to 29% of CC cases ( $p < 0.05$ ).

**Conclusions:** In summary, VMCs are seen far more frequently in patients with CC (intrahepatic and hilar) compared to control group. Presence of pre-neoplastic and transitional lesions in such cases supports the idea that VMCs may represent a precursor of sporadic CC and patients with numerous VMCs are likely at a higher risk of developing CC. Further studies are needed to understand the natural history of these lesions.

**Disclosure of Interest:** None Declared

## The positive effect of exosomes on epithelial-mesenchymal transition through hedgehog signalling in cholangiocarcinoma cells

Ahmet Canbazoglu<sup>1</sup>, Ozlem Kucukoglu<sup>\*1</sup>, Jan-Peter Sowa<sup>1</sup>, Guido Gerken<sup>1</sup>, Bernd Giebel<sup>2</sup>, Ali Canbay<sup>3</sup> and AG Canbay

<sup>1</sup>Department of Gastroenterology and Hepatology, <sup>2</sup>Institute for Transfusion Medicine, University Duisburg-Essen, Essen, <sup>3</sup>Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University, Magdeburg, Germany


**Corresponding author's email:** ozlem.kucukoglu@uni-deu.de

**Introduction:** Cholangiocarcinoma (CCC) is unknown malignancy of epithelial biliary cells and has poor prognosis with less than 5% survival rate at 5-years. Exosomes, cell-derived vesicles, have important roles in cellular trafficking. To understand the role of exosomes on development and metastasis of CCC may lead us to improve the treatments.

**Aims:** In the present study the role of exosomes between healthy and carcinogenic biliary cells and Hedgehog signalling in EMT/MET regulation were analysed.

**Material and Methods:** Exosomes released from healthy human cholangiocyte and cholangiocarcinoma cells were isolated by ultracentrifugation. The particle size distribution was obtained by Nanoparticle Tracking Analysis (NTA). Each cell lines were treated with exosomes released from both cell lines separately. Intakes of exosomes by cells were visualised under confocal super-resolution microscope (Zeiss, LSM710). Cell proliferation (MTT assay) and the gene expression levels of the EMT, Hedgehog signalling and cell death markers were analysed by QPCR as well as by immunoblotting.

**Results:** While exosome secretion of TFK-1 cells were 2.5 fold higher than H69 cells, the treatment of TFK-1 cells with H69 originated exosomes resulted significant reduction of exosome release ( $p < 0.05$ ). Contrarily TFK-1-exosomes treated H69 cells released 1.5 fold more EVs than without treatment. TFK-1 cell proliferation was repressed by H69-EVs. The expressions of epithelial cell marker E-cadherin, death receptor markers (DR4, TRAIL) increased in H69-Evs treated TFK-1 cells ( $p$  values: n.s., 0.03, 0.09, respectively), while N-cadherin, sHh markers (Gli, PTCH1), the necrosis marker HMGB1 was reduced in H69 cells treated with TFK-1-exosomes were reduced ( $p$  values 0.09, 0.07, n.s., 0.002; respectively). In contrast the expressions of E-cadherin, DR4 and Trail decreased in TFK-



1-exosomes treated H69 cells (p values 0.04; 0.03, n.s.; respectively), while the expression of the mesenchymal cell markers (N-cadherin, S100A4) and PTCH1 were reduced (p values 0.001, 0.01, n.s., respectively).

**Conclusions:** Exosomes of cholangiocarcinoma cells alter gene expression of cholangiocytes towards apoptosis resistance and an EMT-like gene expression. Conversely, exosomes of healthy cholangiocytes increase death receptor expression in cholangiocarcinoma cells. The development, metabolism and metastasis of CCC are affected by exosomes and exosomes of healthy cells may be promising treatment option for CCC patients in the future. However, further studies are needed.

**Disclosure of Interest:** None Declared

## The autophagy marker predicts tumor recurrence in intrahepatic cholangiocarcinoma patients after surgical resection

**Chin-Wen Lin<sup>\*1,2</sup>, Gin-Ho Lo<sup>3</sup>, Chia-Chang Hsu<sup>4</sup>, Kah Wee Koh<sup>4</sup>, Pei-Min Hsieh<sup>5</sup>, Yaw-Sen Chen<sup>5</sup> and Division of Gastroenterology and Hepatology, E-Da Dachang Hospital, I-Shou University, Kaohsiung**

*<sup>1</sup>Internal Medicine, E-Da Dachang hospital, I-Shou University, <sup>2</sup>School of Medicine, I-Shou University, <sup>3</sup>Internal Medicine, <sup>4</sup>E-DA hospital, Kaohsiung City, Taiwan, <sup>5</sup>Surgery, E-DA hospital, Kaohsiung City, Taiwan*

**Corresponding author's email:** lincw66@gmail.com


**Introduction:** Intrahepatic cholangiocarcinoma (ICC) is a malignant tumor originating from the epitheliocytes of bile ducts. ICC is the second common primary cancer of the liver and has reported in the adult of USA. Recent studies suggest its incidence is rising worldwide. Surgery is the only modality shown to prolong survival. One- and 5-year survival rates in those with unresectable disease are reported to be 23% and 3%, respectively. However, overall survival rates of 70–80% at 1 year and 30–35% at 5 years after surgical resection have been reported and poor prognosis.

Autophagy plays an important role in the physiology and pathogenesis of human liver diseases. Recently, the autophagy markers show the controversial results in tumor recurrence in HCC patients after hepatectomy but not in ICC. However, these factors are not uniform predict of tumor recurrence of ICC following hepatic surgery.

**Aims:** The study aims to explore that the identification of predicting factors after surgical resection provides a promising strategy to predict of tumor recurrence of ICC patients.

**Material and Methods:** We retrospectively analyzed 88 patients diagnosed with ICC by histology after surgical resection at E-DA hospital/ I-Shou University, Kaohsiung, Taiwan, from 2009 to 2015. The demographic data, recurrence, and survival were collected until December 2016. The expression of autophagy-related markers (LC3, Beclin-1, and p62) were analyzed by IHC staining using non-tumor tissues.





**Results:** Eighty-eight ICC patients underwent surgical resection were collected. The average age is 60.1 years old (range 38-82 years). Fifty-two (59%) patients were male and the median length of follow-up was 5 years. The rate of HBV, HCV, Non-HBVHCV, and HBV/HCV is 45.4%, 28.4%, 23.9%, and 2.3% respectively. Seventy-one patients were recurrence after surgical resection. The negative expression of LC3 in tumor part, microvascular invasion, multiple tumors, negative resection margin, and positive lymph nodes ( $P < 0.001$ ) were significantly associated with increased tumor recurrence in univariate analyses. The negative expression of LC3 in tumor, negative resection margin, and positive lymph nodes were significantly associated with increased tumor recurrence in multivariate analyses ( $P < 0.0001$ ).

**Conclusions:** The autophagy marker LC3 in tumor is a strongly predictive factor related to tumor recurrence in ICC patient who underwent surgical resection.

**Disclosure of Interest:** None Declared

## Liver transplantation in patients with hilar cholangiocarcinoma- single center experience

**Petra Dinjar Kujundžić<sup>\*1</sup>, Ivan Ledinski<sup>2</sup>, Ana Ostojić<sup>1</sup>, Nikola Sobočan<sup>1,2</sup>, Slavko Gašparov<sup>1,2</sup>, Branislav Kocman<sup>1</sup>, Stipislav Jadrijević<sup>1</sup>, Danko Mikulić<sup>1</sup>, Tajana Filipec Kanižaj<sup>1,2</sup>**

<sup>1</sup>Clinical Hospital Merkur, <sup>2</sup>School of Medicine, University of Zagreb, ZAGREB, Croatia

**Corresponding author's email:** Petra.dinjar@gmail.com


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**Introduction:** Liver transplantation (LT) in patients with hilar cholangiocarcinoma (hCCC) is a controversial method of treatment. However, in selected number of patients provides acceptable results.

**Aims:** The aim of this study is to present the outcome of liver transplantation in patients with hCCC in our Center.

**Material and Methods:** A retrospective analysis was performed on data of transplanted patients due to hCCC between 2010 and 2017 in Clinical Hospital Merkur, Croatia. Data were evaluated regarding demographic, clinical, etiological, histopathologic stage, waiting time (WT) on list and outcome.

**Results:** In total period 784 liver transplants were performed, of which 3.4% (n=27) were made due to hCCC. Mean age was  $61 \pm 7.98$  years and majority of patients were male (51.9%). 65.2% of patients were exposed to endobiliary drainage before LT. Primary sclerosing cholangitis was present in only one patient, while the majority of patients (96.3%) did not have associated liver disease. The median time on waiting list was very short and amounted 16.22 days (0-89). The majority of patients were Bismuth type III (44%), followed by type II (24%). On the histopathological examination of explanted liver 65% of cases showed tumor size less than 30 mm. Comparison of tumor size greater and less than 30 mm showed equal shares of lymphovascular invasion, perineural invasion, the invasion of surrounding fatty tissue and early tumor recurrence. The Mayo Clinic protocol of neoadjuvant chemoradiation was not performed. Median follow-up after LT was 16.7 months (range from 0.2 to 73.8). Overall tumor recurrence was 26.1%. There wasn't significant difference in tumors size, lymphovascular invasion, perineural invasion and the invasion of surrounding fatty tissue between group with or without hCCC recurrence. 1- and 3-year survival rate after transplantation was 21/27 (77.7%) and 15/27 (55.5%),



respectively; with 55.6% of death rate due to recurrence of hCCC and 44.4% rate due to postoperative mortality. Only four patients live longer than five years.

**Conclusions:** Orthotopic liver transplantation might be applicable for a highly selected subgroup, emphasizing the need for better adjuvant and neoadjuvant strategies. To define prognostic factors for hCCC recurrence larger cohort of patients need to be analyzed. Current high donor rate in Croatia provides hCCC patients short WT on the list for LT.

**Disclosure of Interest:** None Declared

## Liver progenitor cells significantly contribute to hepatocyte pool in chronic liver injury and cirrhosis: a kinetic study in mice

Rita Manco<sup>\*1</sup>, Laure-Alix Clerbaux<sup>1</sup>, Isabelle Leclercq<sup>1</sup>

<sup>1</sup>Université Catholique de Louvain, Brussels, Belgium


Corresponding author's email: rita.manco@uclouvain.be

**Introduction:** Self-renewal of mature hepatocytes supports homeostasis and regeneration of adult liver. Recent studies indicate that liver progenitor cells (LPC) are recruited upon injury as a facultative reservoir for generation of hepatocytes, although only a small number of mature hepatocytes were shown to derive from LPC *in vivo*. Models used for these studies do not recapitulate long lasting chronic hepatocellular damage and fibrosis seen in human chronic liver disease and cirrhosis.

**Aims:** Our aim is therefore to follow the dynamics of ductular reaction (DR) and the LPC's fate during chronic liver injury in mice.

**Material and Methods:** We used tamoxifen-inducible Osteopontin-Cre (OPN-CreER<sup>T2</sup>) mice crossed with yellow fluorescent protein (YFP) reporter mice to follow the fate of LPC /biliary cells with an efficiency >85%. Long-term chronic injury was induced by repeated carbon tetrachloride (CCl<sub>4</sub>) injections 3x/week for 4, 6, 8, 16 and 24 weeks, resulting in chronic fibrosis and eventually cirrhosis.

**Results:** After 4 weeks CCl<sub>4</sub>, DR is minimal with few ck19<sup>+</sup>/YFP<sup>+</sup> positive cells in periportal area and LPC-derived hepatocytes (traced as YFP<sup>+</sup> hepatocytes) are inconspicuous. After 6 weeks, DR is similar in intensity but small foci of YFP<sup>+</sup> hepatocytes adjacent to portal area are readily seen; these have a median size of 3010 μm<sup>2</sup>. As fibrotic disease increases in severity, the DR is negligible while patches of YFP<sup>+</sup> hepatocytes become larger (median size of 3850 μm<sup>2</sup> and 7040 μm<sup>2</sup> at 8 and 16weeks, respectively) and extend to into the parenchyma. In the cirrhotic liver (24 weeks CCl<sub>4</sub>) some regenerative nodules are entirely composed of YFP<sup>+</sup> hepatocytes. The number of YFP<sup>+</sup> hepatocytes does not rise accordingly to the size of the patches as they represent 4.2 ± 2.4% of the lobule area in 6 weeks' samples, increases up to 11.5 ± 3.8 % in 8 weeks' samples and stabilizes around 5% thereafter, suggesting that not all YFP<sup>+</sup> hepatocytes expand into growing patches. At 6 weeks, YFP<sup>+</sup> hepatocytes are significantly smaller cells than YFP<sup>-</sup> native hepatocytes



(750 vs. 981  $\mu\text{m}^2$ ) but in 16 weeks' samples YFP<sup>+</sup> and YFP<sup>-</sup> hepatocytes have the same size (996 and 1001  $\mu\text{m}$ ).

**Conclusions:** Our data demonstrate a significant contribution of LPC-driven regeneration in a model of chronic liver injury leading to cirrhosis. The kinetic study supports that when DR is present, LPC differentiate into small hepatocytes, some of these subsequently increase in number and in size. Clonality studies are ongoing to test this hypothesis.

**Disclosure of Interest:** None Declared

## Preliminary results from application of image processing tools and quantitative analysis of MRCP datasets of various aetiologies

Siddharth Vikal<sup>1</sup>, Andy McKay<sup>\*1</sup>, Nassim Parvizi<sup>2</sup>, Joana G. E. Leal<sup>3</sup>, Matteo Milanese<sup>1</sup>, Michael Brady<sup>1</sup>, Rajarshi Banerjee<sup>1</sup>

<sup>1</sup>Perspectum Diagnostics Ltd, <sup>2</sup>Radiology, Oxford University Hospital NHS Trust, <sup>3</sup>Oxford Centre for Magnetic Resonance Research, Oxford, United Kingdom

**Corresponding author's email:** sid.vikal@perspectum-diagnostics.com

**Introduction:** MRCP is used extensively in the evaluation of gall bladder anatomy, biliary structures, chronic pancreatitis and a range of hepato-biliary disorders. We have developed image processing methods for enhancement, visualization, and automatic quantification of biliary structures. We precisely measure the bile duct centre line, cross-sectional width, orientation, and the curvature at each point of each bile duct. We measured the Common Bile Duct (CBD), Left Hepatic Duct (LHD) and Right Hepatic Duct (RHD).

**Aims:** Four hypotheses were tested for predictors of healthy vs diseased: 1. Mean CBD diameter distribution; 2. Asymmetry of LHD and RHD diameters; 3. LHD and CBD diameter difference; 4. RHD and CBD diameter difference.

**Material and Methods:** MRCP (Siemens TrioTim 3T, respiratory gated, TR=3300ms, TE=705ms, isotropic voxels size 1.25mm, no contrast agent) was acquired for 101 patients with a variety of liver diseases, and 8 healthy volunteers.

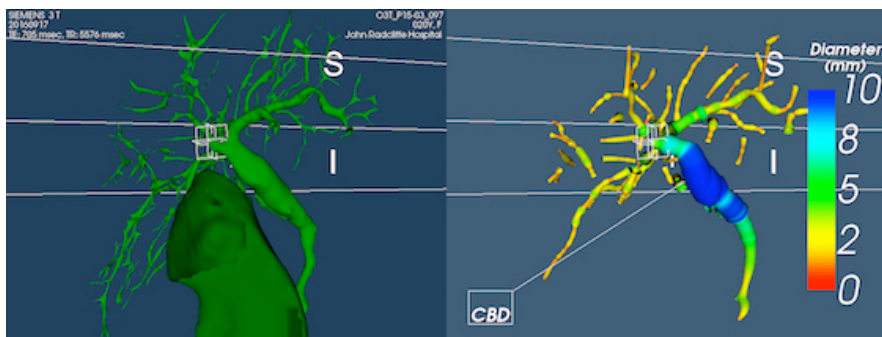
The enhancement of biliary structures is based on analysis of the image second derivatives (Hessian), implemented as a post-processing step. We have further developed analytical methods to quantitatively model (Figure 1) the biliary ducts, yielding precise measurements of the bile duct centre line, cross-sectional width, orientation, and the curvature at each point of each bile duct. In this study, we analysed the MRCP datasets recording measurements of the Common Bile Duct (CBD), Left Hepatic Duct (LHD) and Right Hepatic Duct (RHD). We performed one-way analysis of variance (ANOVA) for various measurements across 3 groups (Healthy, AIH, and PSC/PBC).

**Results:** We report results for 27 patients analyzed so far: 13 with AIH, 6 with PBC, and 8 with PSC, as well as the 8 healthy volunteers. We divided the data into 3 groups: healthy

volunteers, biliary disorders (PSC and PBC), and (non-biliary) AIH. The CBD diameter was: healthy volunteers was  $2.9 \pm 0.4$  mm; AIH  $3.6 \pm 0.8$  mm; PSC/PBC  $4.8 \pm 1.8$  mm. The LHD diameter was: healthy volunteers  $2.4 \pm 0.3$  mm; AIH  $2.6 \pm 0.4$  mm; PSC/PBC  $2.8 \pm 0.7$  mm. The RHD diameter was: healthy volunteers  $2.1 \pm 0.3$  mm; AIH  $2.4 \pm 0.3$  mm; PSC/PBC  $2.6 \pm 0.4$  mm. The differences in CBD diameters is statistically significant ( $p=0.006$ ); differences in LHD-CBD (RHD-CBD) are weakly significant ( $p=0.04$ ,  $p=0.03$ ). LHD-RHD asymmetry does not appear to be significant ( $p=0.54$ ).

**Conclusions:** Quantitative image analysis of MRCP enables measurement of a range of aspects of biliary ducts, enabling objective analysis and informing diagnosis of a range of disease etiologies.

**Figure:**



**Disclosure of Interest:** None Declared

## Common variant p.D19H of the hepatobiliary sterol transporter *ABCG8* is associated with increased gallstone risk and distorted sterol homeostasis in children

Marcin Krawczyk<sup>1,2</sup>, Olga Niewiadomska<sup>3</sup>, Krzysztof Jankowski<sup>4</sup>, Irena Jankowska<sup>3</sup>, Susanne N. Weber<sup>1</sup>, Jolanta Gozdowska<sup>5</sup>, Dariusz Lebensztejn<sup>6</sup>, Sabina Wiecek<sup>7</sup>, Dieter Lütjohann<sup>8</sup>, Piotr Socha<sup>3</sup>, Frank Lammert<sup>1</sup>

<sup>1</sup>Department of Medicine II, Saarland University Medical Center, Saarland University, Homburg, Germany, <sup>2</sup>Laboratory of Metabolic Liver Diseases, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, <sup>3</sup>Department of Gastroenterology, Hepatology and Nutrition Disorders, The Children's Memorial Health Institute, <sup>4</sup>Department Internal Medicine and Cardiology, Medical University of Warsaw, <sup>5</sup>Department Transplantation Medicine and Nephrology, Medical University of Warsaw, Warsaw, <sup>6</sup>Department of Pediatrics, Gastroenterology and Allergology, Medical University of Białystok, Białystok, <sup>7</sup>Department of Pediatrics, Silesian Medical Academy, Katowice, Poland, <sup>8</sup>Institute of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Bonn, Germany


**Corresponding author's email:** marcin.krawczyk@uks.eu

**Introduction:** Gallstone risk factors are believed to differ in children and in adults. In children black pigment stones are often suspected, whereas in adults stones consist mainly of cholesterol. Genetic studies demonstrated that “adult” stones are more frequent in carriers of the *ABCG8* p.D19H variant. The *ABCG5/8* heterodimer functions as hepatocanicular cholesterol transporter. The rate of cholesterol absorption and synthesis can be measured by quantification of serum phytosterols (plant sterols) and cholesterol precursors, respectively.

**Aims:** Here we assess the effects of the p.D19H variant on gallstone risk and cholesterol homeostasis in children with gallstones.

**Material and Methods:** Overall, we recruited 214 children with gallstone disease (age 1 month-17 years, 107 males). Symptomatic stones were present in 138 patients, 47 underwent cholecystectomy, ERCP was required in 10 cases; 126 children received ursodeoxycholic acid. The control gallstone-free cohort comprised 172 adults (age 40-92 years, 70 males). The *ABCG8* p.D19H variant was genotyped using TaqMan assays. Serum concentrations of plant sterols and cholesterol precursors were measured using gas chromatography/mass spectrometry (GC/MS) in 52 children with gallstones.





**Results:** The *ABCG8* risk allele was more frequent in children with gallstones (14.9%) than in controls (7.5%). Presence of the lithogenic genotype was associated with an increased risk of stones (common OR=1.82, P=0.03). Notably, even carriers of one copy of the lithogenic allele demonstrated increased gallstone prevalence (OR=2.18, 95% CI=1.08-4.40, P=0.02). Patients carrying the lithogenic allele displayed lower serum concentrations of the natural phytosterol sitosterol (P=0.04) and decreased serum phytosteranols, i.e. campestanol (P=0.02) and sitostanol (0.02). In line with these findings, the *ABCG8* p.D19H variant was associated with decreased ratios of phytosterols to cholesterol precursors (sitosterol:desmosterol, P <0.01; campesterol:desmosterol, P=0.01).

**Conclusions:** Children carrying the frequent *ABCG8* variant p.19H are, alike adults, at-risk of gallstones. This is most likely due to increased cholesterol synthesis in response to higher sterol clearance present already at the young age. Overall, cholesterol gallstones might be more frequent in paediatric patients than estimated previously. This might be due to an inherited lifelong lithogenic predisposition for which precise therapeutic strategies need to be developed.

**Disclosure of Interest:** None Declared

## The anti-inflammatory receptor TREM-2 protects the liver from cholestatic injury in mice

Aitor Esparza-Baquer<sup>\* 1</sup>, Ibone Labiano<sup>1,2</sup>, Álvaro Santos-Laso<sup>1</sup>, Oihane Erice<sup>1</sup>, Ander Arbelaiz<sup>1</sup>, Omar Sharif<sup>6, 4</sup>, Elizabeth Hijona<sup>1</sup>, Raúl Jiménez-Agüero<sup>1</sup>, Marco Marzioni<sup>5</sup>, Derek A. Mann<sup>6</sup>, Luis Bujanda<sup>1,2</sup>, Jesús M. Banales<sup>1,2,7</sup>, María J. Perugorria<sup>1,2,7</sup>

<sup>1</sup>Department of Liver and Gastrointestinal Diseases, Biodonostia Health and Research Institute, San Sebastián, <sup>2</sup>National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Carlos III National Health of Health, Madrid, Spain, <sup>3</sup>Department of Medicine I, Laboratory of Infection Biology, Medical University of Vienna, <sup>4</sup>Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria, <sup>5</sup>Department of Gastroenterology and Hepatology, Università Politecnica delle Marche, Ancona, Italy, <sup>6</sup>Institute of Cellular Medicine, Faculty of Medical Sciences, 4th Floor, William Leech Building, Newcastle University, Newcastle upon Tyne, United Kingdom, <sup>7</sup>IKERBASQUE, Basque Foundation for Science, Bilbao, Spain


**Corresponding author's email:** aitor.esparza@biodonostia.org

**Introduction:** Cholestasis is a common feature of different cholangiopathies such as PSC and PBC. Cholestasis causes liver inflammation and injury in epithelial cells, thereby activating non-parenchymal liver cells [i.e. kupffer cells (KC) and hepatic stellate cells (HSC)] that promote wound-healing responses that ultimately result in ductular reaction and biliary fibrosis. Impaired bile flow leads to alterations in the intestine epithelial barrier, enabling the translocation of bacterial components to the liver via the portal vein. In the liver, these bacterial components bind to toll-like receptors (TLRs) expressed in KC and HSC promoting inflammation and progression of the wound-healing response. The triggering receptor expressed on myeloid cells-2 (TREM-2) is an anti-inflammatory receptor that inhibits TLR-mediated signaling.

**Aims:** This study aims to evaluate the role of TREM-2 in cholestasis.

**Material and Methods:** Wild type (WT) and *Trem-2* knock out (*Trem-2*<sup>-/-</sup>) mice were subjected to bile duct ligation (BDL), or sham, for 7 days. Thereafter, sera were collected for the analysis of biochemical markers and livers were obtained for further histological and gene expression analysis. *In vitro*, KC were isolated from WT and *Trem-2*<sup>-/-</sup> mice, treated with lipopolysaccharide (LPS) and cytokine and chemokine expression was assessed.

**Results:** TREM-2 is specifically expressed by non-parenchymal cells but is not present



in epithelial cells. *Trem-2* mRNA levels were upregulated in the liver of WT animals after BDL. In BDL mice, *Trem-2*<sup>-/-</sup> showed exacerbated liver injury with increased hepatocyte necrosis and immune-cell infiltration compared to WT, as assessed by HandE staining. This was accompanied by augmented mRNA levels of cholangiocyte (CK-7 and CK-19) and proliferation markers (Ki67 and PCNA), indicating that *Trem-2*<sup>-/-</sup> animals suffered exacerbated ductular reaction. Likewise, increased Sirius Red staining, as well as Col1a1 and aSMA mRNA levels, revealed enhanced fibrogenesis in *Trem-2*<sup>-/-</sup> livers compared to WT after BDL. In addition, the expression of pro-inflammatory cytokines (IL-6 and TNF- $\alpha$ ) and chemokines (MCP-1 and CXCL1) were upregulated in these mice. LPS-treated *Trem-2*<sup>-/-</sup> KC displayed increased expression of pro-inflammatory and chemokine markers, both at mRNA and protein level, as compared to WT-derived KC.

**Conclusions:** TREM-2 is overexpressed during experimental cholestasis and it negatively regulates TLR4-mediated pro-inflammatory cytokine expression in KC, thereby protecting the liver from cholestatic injury in mice.

**Disclosure of Interest:** None Declared

## CD86<sup>+</sup>/CD206<sup>+</sup> tumor-associated macrophages predict prognosis of patients with intrahepatic cholangiocarcinoma

Dalong Sun<sup>\*1</sup>, Longzi Liu<sup>1</sup>, Xiaoxiao Wang<sup>2</sup>, Guangxi Zhao<sup>1</sup>, Pingping Dong<sup>1</sup>, Ling Dong<sup>1</sup>

<sup>1</sup>Zhongshan Hospital, Fudan University, <sup>2</sup>Department of Biochemistry and Molecular Biology, Fudan University, Shanghai, China


Corresponding author's email: sdlsu@aliyun.com

**Introduction:** As the main cellular ingredients of tumor microenvironment, tumor-associated macrophages (TAMs) play a vital role in tumor development and progression. Recently, some studies have suggested that TAMs are sensitive and specific prognostic factors in numerous cancers. However, the profile of TAMs alteration and its correlation with ICC prognosis were uncertain.

**Aims:** The primary purpose of this study is to determine the prognostic significance of TAMs in intrahepatic cholangiocarcinoma (ICC).

**Material and Methods:** The immunohistochemical staining of CD68, CD86 and CD206 were performed in tissue microarrays containing 322 patients who had undergone surgical resection and were pathologically diagnosed with ICC. The prognostic value of CD68, CD86 and CD206 were evaluated by Kaplan–Meier analysis (log-rank test) and nomogram models.

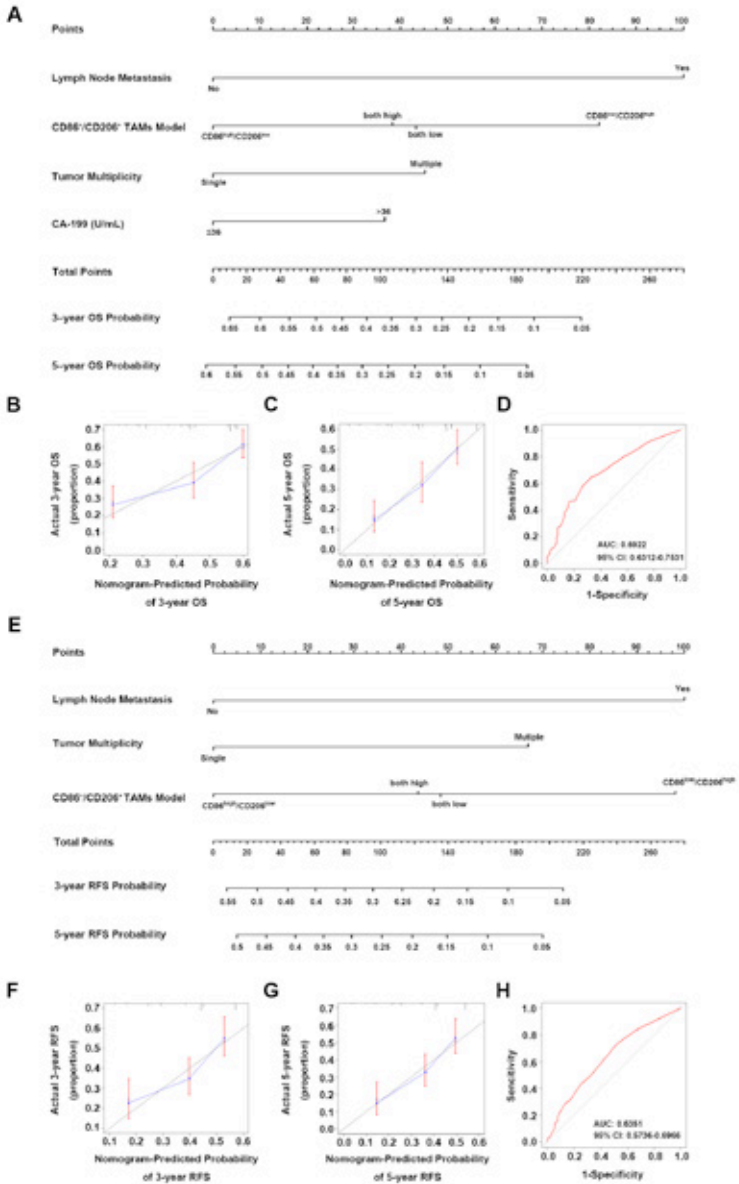
**Results:** We demonstrated that CD86<sup>+</sup>/CD206<sup>+</sup> TAMs model was an independent prognostic index for ICC patients. The patients with low CD86<sup>+</sup> TAMs and high CD206<sup>+</sup> TAMs infiltration had a markedly worse prognosis and increased risk of post-operation recurrence when compared with high CD86<sup>+</sup> TAMs and low CD206<sup>+</sup> TAMs intratumoral infiltration, respectively. Furthermore, subgroup analysis indicated that CD86<sup>+</sup>/CD206<sup>+</sup> TAMs model predicted prognosis of ICC patients more powerfully than single macrophages immune marker. Interestingly, CD86<sup>+</sup>/CD206<sup>+</sup> TAMs model could further distinguish CA-199 negative ICC patients, who were generally supposed with favorable prognosis. In order to further perfect the prognostic value of CD86<sup>+</sup>/CD206<sup>+</sup> TAMs model, we constructed and validated a postoperative nomogram to predict overall survival (OS) and recurrence-free survival time (RFS) in ICC patients. Internal validation



of the nomograms was carried out by calibration plots with bootstrap sampling ( $m = 100$ ,  $n = 1000$ ). The C-indexes for the OS and RFS nomograms were 0.649 and 0.635, respectively. Both calibration plots were closely relative to the 45-degree line. Additionally, the ROC was performed to further evaluate the nomograms. The AUC was 0.6922 (95% CI: 0.6312-0.7531) for the OS nomogram and 0.6351 (95% CI: 0.5736-0.6966) for the RFS nomogram.

**Conclusions:** Combined analysis of CD86<sup>+</sup>/CD206<sup>+</sup> TAMs has a better prognostic value than individual analysis for ICC survival and recurrence, especially in CA-199 negative patients. Our findings provide promising targets for future investigation or intervention of ICC.

**Figure:**



**Disclosure of Interest:** None Declared

## Fluoroscopically guided biopsies do not seem to improve cancer detection rates in patients with biliary strictures undergoing brush cytology during ERCP

**Daniela Reis\*<sup>1</sup>, Carlos Noronha Ferreira<sup>1</sup>, Carlos Freitas<sup>1</sup>, António Marques<sup>1</sup>, Rosário Maldonado<sup>1</sup>, Rui Palma<sup>1</sup>, Luís Carrilho-Ribeiro<sup>1</sup>, José Velosa<sup>1</sup>**

*<sup>1</sup>Gastroenterology and Hepatology, Hospital de Santa Maria, North Lisbon Hospital Centre, Lisbon, Portugal*

**Corresponding author's email:** [daniela.ant.reis@gmail.com](mailto:daniela.ant.reis@gmail.com)

**Introduction:** Brush cytology during ERCP has a low sensitivity for cancer detection in biliary strictures. Biopsies of strictures under fluoroscopic guidance may improve cancer detection.

**Aims:** The aim of this study was to assess diagnostic yield and accuracy of endoscopic brush cytology during ERCP and to verify if biopsies improve the detection of malignant biliary strictures.

**Material and Methods:** We reviewed 168 consecutive patients with biliary strictures who underwent endoscopic brush cytology with or without biopsies, between January 2010 and July 2016. Brush cytology was performed at the site of biliary stricture at least 10 times. Biopsies were done according to the decision of the endoscopist performing ERCP and only in strictures involving the main bile duct (MBD). At least two fragments were obtained by biopsy for histopathological diagnosis. Final diagnosis was based on brush cytology, surgery, EUS, percutaneous biopsy or clinical follow-up.

**Results:** Out of 168 patients studied, 18 were excluded due to incomplete clinical data. The mean age of 150 patients analyzed was 69.25 (16-91) years and 91 were males. Single stenosis of the MBD was documented in 144 (96%) patients. In 81 (54%) patients, the stenosis was located in the distal MBD. Biopsies were performed in 79 (53%) patients.

Finally, 104 patients had a malignant biliary stricture with pancreatic cancer (n=49) and cholangiocarcinoma (n=42) being the most common etiologies. Brush cytology alone had a sensitivity of 37.3% (95% CI, 27.88-47.39%) and specificity of 97.02% (95% CI, 88.93-99.95%), and an accuracy of 56.7% in the diagnosis of malignant biliary stenosis.

A combination of brush cytology and biopsies yielded only slight increase in sensitivity to 39.42%, (95% CI, 29.98-49.49%), specificity of 97.8%, (95% CI 87.03-99.88) with an accuracy of 57.33%.

**Conclusions:** Our study results suggest that a combination of brush cytology and biopsies during ERCP does not improve the characterization of malignant biliary strictures compared to brush cytology alone. This lack of benefit of fluoroscopically guided biopsies in our study sample may have been due to the high prevalence of pancreatic cancer as the cause for malignant biliary strictures.

**Disclosure of Interest:** None Declared



## Hepatoprotective effect of S-Adenosyl L-Methionine (SAME) in oxidative stress: in vitro study on hepatocytes and cholangiocytes

Ewa Kilanczyk<sup>\*1</sup>, Piotr Milkiewicz<sup>2,3</sup>, Jesus M. Banales<sup>4</sup>, Malgorzata Milkiewicz<sup>1</sup>

<sup>1</sup>Department of Medical Biology, MCD1, Pomeranian Medical University, Szczecin, <sup>2</sup>Liver and Internal Medicine Unit, Medical University of Warsaw, Warsaw, <sup>3</sup>Translational Medicine Group, Pomeranian Medical University, Szczecin, Poland, <sup>4</sup>Department of Liver and Gastrointestinal Diseases, Biodonostia Research Institute-Donostia University Hospital-Ikerbasque, CIBERehd, University of the Basque Country, Sa Sebastian, Spain

**Corresponding author's email:** ewakilanczyk@gmail.com

**Introduction:** S-Adenosyl-l-methionine (SAME) is involved in important detoxifying processes including trans-sulphuration and trans-methylation. Acquired deficiency of SAME synthetase leading to decreased levels of SAME is characteristic of several chronic conditions. Several reports suggest the role of SAME as a potential hepatoprotective agent.

**Aims:** The aim of this study was to check the particular role of SAME on the hepatobiliary oxidative stress.

**Material and Methods:** Oxidative stress was experimentally induced in hepatocellular carcinoma cells (HEp-G2) and normal human cholangiocytes (NHC) using H<sub>2</sub>O<sub>2</sub> (250 μM for HEp-G2; 100 mM for NHC) and TBHQ (500 μM for HEp-G2; 30 μM for NHC). Cells were co-treated with different concentrations of SAME (10, 50, 100 and 200 μM) followed by MTT Tetrazolium Assay in order to evaluate cell viability. Additionally, to find out whether a potential protection with SAME depends on the level of reduced glutathione, glutathione synthetase inhibitor "buthioninesulfoximine" (BSO, 100μM) was applied. The protein level of Nrf-2 (NE-F2 related factor), i.e. marker of oxidative stress, was measured (Western blot) in HEp-G2 cells after exposure to H<sub>2</sub>O<sub>2</sub> (500μM, 2h) with or without pre-treatment with SAME (100 μM, 30 min. before H<sub>2</sub>O<sub>2</sub>) or BSO (100 μM, 16h before H<sub>2</sub>O<sub>2</sub>).

**Results:** H<sub>2</sub>O<sub>2</sub> and TBHQ significantly reduced the viability of HEp-G2 and NHC cells (~40% reduction). All doses of SAME protected HEp-G2 and NHC cells against the oxidative stress induced by either H<sub>2</sub>O<sub>2</sub> or TBHQ. The level of Nrf-2 was significantly

lower in Hep-G2 cells after treatment with SAME alone, and pre-treatment with SAME diminished the H<sub>2</sub>O<sub>2</sub>-induced increased levels of Nrf-2. Furthermore, SAME protection was not abolished by BSO.

**Conclusions:** This study is the evidence that human hepatocytes and cholangiocytes under oxidative stress may be protected by the treatment with SAME and that SAME was able to lowered levels of Nrf-2 in HEp-G2 cells and finally to inhibit oxidative stress. Interestingly, SAME anti-oxidant defense does not involve reduced glutathione (GSH) since GSH inhibitor cannot hinder this process. These data suggest that SAME may provide potential clinical benefits in chronic liver conditions.

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**Disclosure of Interest:** None Declared

## Anti-apoptotic role of c-FLIP in human cholangiocarcinoma: activation of Fas/FasL pathway

Gianluca Carnevale\* <sup>1</sup>, Guido Carpino<sup>2</sup>, Vincenzo Cardinale<sup>3</sup>, Alessandra Pisciotta<sup>1</sup>, Lorenzo Nevi<sup>3</sup>, Sara De Biasi<sup>1</sup>, Lara Gibellini<sup>1</sup>, Andrea Cossarizza<sup>1</sup>, Eugenio Gaudio<sup>4</sup>, Anto de Pol<sup>1</sup>, Domenico Alvaro<sup>3</sup>

<sup>1</sup>*Surgery, Medicine, Dentistry and Morphological Sciences, University of Modena and Reggio Emilia, Modena*, <sup>2</sup>*Movement, Human and Health Sciences, University of Rome "Foro Italico"*, <sup>3</sup>*Medico-Surgical Sciences and Biotechnologies*, <sup>4</sup>*Anatomical, Histological, Forensic Medicine and Orthopedics Sciences, Sapienza University of Rome, Rome, Italy*

**Corresponding author's email:** gianluca.carnevale@unimore.it

**Introduction:** Cholangiocarcinoma (CCA) comprises a heterogeneous group of malignancies characterized by biologic aggressiveness and treatment refractory nature.

**Aims:** The aim of the study was to investigate the role of Fas/FasL pathway and the effects of human immune cells on proliferation and apoptosis of human intrahepatic CCA (iCCA).

**Material and Methods:** The expression of Fas, Fas-L, FADD, c-FLIP<sub>SL</sub>, Bcl-2, procaspase 8 and caspase 3 was evaluated in situ and in primary cultures from iCCA specimens. Direct co-cultures with peripheral blood mononuclear cells (PBMCs) were used to analyze the influence on CCA cell proliferation and apoptosis as well as to evaluate the apoptotic machinery downstream of Fas/FasL pathway.

**Results:** Fas, FasL, and c-FLIP<sub>SL</sub> expressions were increased in iCCA specimens in comparison with peri-tumoral normal interlobular bile ducts. FasL expression co-localized with stem cell markers in the same cancer cell. Accordingly, primary cultures of stem cell-like subset of CCA cells constitutively expresses Fas and FasL. Following direct co-culture with PBMCs the expression of Fas and FasL significantly increased after 24, 48 and 72 hours and it is paralleled by an augmentation of proliferation rate of the cancer cells. Conversely a significant increase of apoptotic CD4 and CD8 positive T-cells or CD56 positive Natural Killers were observed in co-culture with respect to the PBMCs cultured alone. Western Blot analysis revealed a steady expression of FADD either in iCCA primary cells cultured alone or co-cultured with PBMCs. Interestingly, we observed in CCA cells a significant increase of c-FLIP<sub>SL</sub> expression along the whole co-culture time with PBMCs.

Immunofluorescence analysis showed a strictly nuclear staining for c-FLIP<sub>S/L</sub> in CCA cells cultured alone, whereas after co-culture with PBMCs either a nuclear and a cytoplasmic staining for c-FLIP<sub>S/L</sub> was observed. Interestingly, a significant increase of the expression of pro-caspase 8 and Bcl-2, was detected, while no differences in the expression of cleaved caspase-3 were observed.

**Conclusions:** our data demonstrate that stem cell-like subset of CCA cells increased their expression of Fas and FasL when are in direct contact with PBMCs followed by an increase of c-FLIP<sub>S/L</sub>, thus culminating in anti apoptotic and proliferative effects in cancer cells. Finally, c-FLIP<sub>S/L</sub> seems to represent a key molecule in the processes implicated in the immune-escape of stem cell-like subset of CCA cells and could represent a potential therapeutic target for this cancer.

**Disclosure of Interest:** None Declared

## Insulin-like growth factor signaling axis confers acquired resistance to EGFR targeting drug erlotinib by inducing EMT/CSC in human cholangiocarcinoma cells

Javier Vaquero<sup>1, 2</sup>, Cindy Lobe<sup>1</sup>, Sylvana Tahraoui<sup>1</sup>, Audrey Clapéron<sup>1</sup>, Martine Mergery<sup>1</sup>, Fatiha Merabtene<sup>1, 3</sup>, Dominique Wendum<sup>1, 4</sup>, Cédric Coulouarn<sup>5</sup>, Christèle Desbois-Mouthon<sup>1</sup>, Françoise Praz<sup>1</sup>, Laura Fouassier<sup>\*1</sup>

<sup>1</sup>Sorbonne Universités, UPMC Univ Paris 06, INSERM, Saint-Antoine Research Center, F-75012, Paris, France, <sup>2</sup>Fondation ARC, F-94803, Villejuif, France, <sup>3</sup>Histomorphology Platform, UMS 30 Lumic, F-75012, <sup>4</sup>Department of Pathology, AP-HP, Saint-Antoine Hospital, F-75012, Paris, France, <sup>5</sup>INSERM, INRA, Univ Rennes 1, Univ Bretagne Loire, Nutrition Metabolisms and Cancer (NuMeCan), F-35033, Rennes, France

**Corresponding author's email:** [laura.fouassier@inserm.fr](mailto:laura.fouassier@inserm.fr)

**Introduction:** Cholangiocarcinoma (CCA) is a tumor that displays a biliary epithelial cell phenotype and a desmoplastic stroma with high cancer-associated fibroblasts (CAF) content. CCA has a very poor prognosis due to its late clinical presentation and lack of effective non-surgical therapies. Epidermal growth factor receptor (EGFR) is overexpressed in CCA and contributes to its progression. Thus, EGFR was envisaged as a target for CCA therapy. However, clinical trials using the EGFR tyrosine kinase inhibitor (TKI) erlotinib, did not provide a therapeutic benefit in patients with CCA, suggesting the existence of resistance mechanisms.

**Aims:** This study aimed to unravel the underlying molecular mechanisms involved in resistance to erlotinib in CCA.

**Material and Methods:** Erlotinib-resistant cells were obtained by treating four human CCA cell lines with increasing concentrations of erlotinib for long term. Cell viability was determined by the cristal violet method. Signaling pathways were analyzed by phosphoprotein arrays and WB. BMS-536924 and linsitinib were used to inhibit the insulin/insulin-like growth factor-1 receptors (IR/IGF1R). Cell migration was evaluated by Boyden chamber assay and wound healing assay. Cell clonogenicity was evaluated by colony and sphere formation assays. *In vivo* experiments were performed using a xenograft tumor model in immunodeficient mice.

**Results:** We found an activation of insulin receptor (IR) and insulin-like growth factor (IGF) 1 receptor (IGF1R) in erlotinib-resistant CCA cells, along with an increase of IGF2

expression. Gene set enrichment analysis revealed a metastasis signature in erlotinib-resistant CCA cells. Consistently, a markedly change of cell morphology was observed with a scattered phenotype and an epithelial-mesenchymal transition (EMT) leading to an increase of migratory properties, combined with the acquisition of cancer stem cell (CSC) traits. Inhibition of IR/IGF1R with linsitinib reduced EMT and restored sensitivity to erlotinib of CCA cells both *in vitro* and *in vivo* in nude mouse xenograft model. Likewise, *in vivo* linsitinib reduced the contingent of CAF, which express IGF2 suggesting a role of these cells in CCA resistance to erlotinib. In human CCA, IGF2 and IGF1R are expressed by tumor cells and CAF.

**Conclusions:** Activation of IGF axis supports erlotinib resistance in CCA cells by inducing EMT/CSC. These findings suggest that inhibition of IR/IGF1R in combination with erlotinib could benefit to CCA patients.

**Disclosure of Interest:** None Declared

## Epigenetic profiling of premalignant and malignant stages of cholangiocarcinoma

Ursula Lemberger\*<sup>1</sup>, Walter Pulverer<sup>2</sup>, Milan Geyer<sup>3</sup>, Trine Følseraas<sup>4</sup>, Jochen Hampe<sup>5</sup>, Christoph Österreicher<sup>6</sup>, Gerda Egger<sup>1</sup> and Assoc. Prof. Dr. Gerda Egger

<sup>1</sup>Department of Pathology, Department of Pathology, Medical University Vienna, <sup>2</sup>Molecular Medicine Health and Environment Department, Austrian Institute of Technology GmbH, <sup>3</sup>Department of Pathology, Medical University Vienna, Vienna, Austria, <sup>4</sup>Norwegian PSC Research Center, Department of Transplantation Medicine, Division of Surgery, Inflammatory Medicine and Transplantation, Oslo University Hospital Rikshospitalet, Oslo, Norway, Oslo, Norway, <sup>5</sup>Medical Department 1, University Hospital Dresden, Technische Universität Dresden, Dresden, Germany, <sup>6</sup>Institute of Pharmacology, Medical University Vienna, Vienna, Austria

**Corresponding author's email:** ursula.lemberger@meduniwien.ac.at

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**Introduction:** Primary sclerosing cholangitis (PSC) is a rare cholestatic liver disease, characterized by chronic inflammation and fibrosis of bile ducts leading to biliary cirrhosis. Diagnosis of PSC is complicated by heterogeneous clinical symptoms and represents a considerable risk factor for the development of cholangiocarcinoma (CCA). CCA is characterized by a poor prognosis with less than 5% 5-year survival rates, due to frequent tumor recurrence and limited therapy options. Several genetic mutations have been identified in PSC and CCAs, but defined molecular pathways of tumorigenesis are still unknown and biomarkers to aid early diagnosis to allow tumor classification and patient stratification are missing. Changes in epigenetic patterns have been associated with different forms of chronic liver diseases including cancer. Aberrant DNA methylation is associated with a variety of tumors and represents a promising tool for diagnosis, prognosis and therapy response prediction.

**Aims:** Investigation of the methylome of cholangiocytes in healthy tissue, PSC and CCA patients in order to define diagnostic and prognostic marker.

**Material and Methods:** We analyzed the DNA methylome of patients with PSC, CCA and healthy human liver tissue using Infinium HumanMethylation450 BeadChip. The top differentially methylated CpGs were validated by targeted bisulfite sequencing in a second, larger patient cohort. Class prediction was performed in order to define a set of differentially methylated CpG sites that could discriminate between the different sample groups.

**Results:** Genome-wide DNA methylation analyses displayed a clear separation of normal liver samples, PCS and CCA samples based on their methylation profile. These data suggest that aberrant epigenetic signatures are already present in the premalignant stages of CCA. A set of 80 differentially methylated regions was then tested using bisulfite sequencing in a larger patient cohort, yielding 598 informative CpG sites. We performed compound covariate predictor classification based on 10 markers, which allowed the distinction of all groups tested with high specificity and sensitivity.

**Conclusions:** Aberrant DNA methylation is present in premalignant stages of cholestatic liver disease. Our findings might be of prognostic relevance for patients suffering from PSC. Distinct methylation profiles might be associated with specific genetic lesions in CCA patients, which could be useful for molecular tumor classification.

**Disclosure of Interest:** None Declared





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