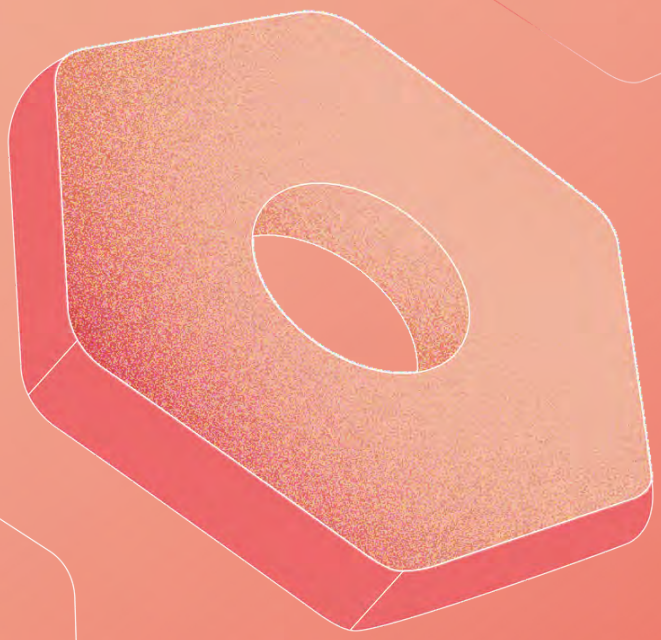
EASL LIVER CANCER SUMMIT 2024

Rotterdam, Netherlands
22–24 February

# ABSTRACT BOOK



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## Content

### ORAL ABSTRACT PRESENTATIONS



### POSTER ABSTRACT PRESENTATIONS - Basic Science

## POSTER ABSTRACT PRESENTATIONS - Clinical Science

# ORAL ABSTRACT PRESENTATIONS

# OS-1
# Histone H1.4 lactylation activates MZF1 to promote hepatocellular carcinoma progression

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**Background and aims:**
Intracellular lactate induced lysine lactylation (Kla) on core histones drives the oncogenic process. In this study, we investigate Kla's regulation on histone H1.4 and its regulation on oncogene MZF1 and HCC progression.

**Method:**
Intersective analysis of ChIP-seq, ATAC-seq, RNA-seq, and snRNA-seq was performed to find Kla target gene in both HCC cell lines and patient samples. MZF1 was sorted out and ChIP PCR was used for verification. *In vitro* and *in vivo* experiments were then constructed to validate the function of Kla and MZF1 on HCC behavior. DNA pull down assay combined with mass spectrometry was used to find the upstream regulator of MZF1. Histone H1.4 was recognized and its direct binding on MZF1 promoter region was recognized by ChIP PCR. RNA-seq and scRNA-seq data were used to search for the downstream pathway of MZF1.

**Results:**
Single-nuclei RNA sequencing was performed profiling tumor biopsy samples from HCC patients with lung metastasis (M1) or without lung metastasis (M0). 14 clusters of HCC cells were identified (fig. 1A). Pathways regulating glucose homeostasis, carbohydrate homeostasis, and regulation of glycolytic process and positive regulation of Wnt signaling pathway were enriched in the M1 group specific clusters (fig. 1B). The effect of glycolysis product lactate and lactate-stimulated Kla was therefore examined. Cell functional assays showed that lactate could enhance cell migration (fig. 1C) and invasion (fig. 1D), while lactate inhibitors inhibited cell function (fig. 1E, 1F). *In vivo* models were constructed and the results were consistent with *in vitro* experiments (fig. 1G- 1L). Multiomic analysis was then performed to reveal the downstream regulation of lactate-stimulated Kla, and the only overlapped target was MZF1 (fig. 1M, 1N). WB results showed that MZF1 increased after lactate and glucose treatment, and decreased after treated with OXA and DCA in HCC cell lines (fig. 1O, 1P) and *in vivo* models (fig. 2B, 2C). CUT and Tag qPCR validated the binding of Kla at the promoter region of MZF1 (fig. 2A). Mass spectrum results recognized histone H1.4 as a direct binding protein of MZF1 DNA (fig. 2D). CUT and Tag qPCR on mutated H1.4 residues confirmed the binding at the promoter region of MZF1, and mutation of K90 residue was most significant (fig. 2E). Enrichment analysis indicated that Wnt signaling was enriched in the 136 differential genes (fig. 2F, 2G). This was confirmed by WB results (fig. 2H. 2I) of lactate and/or FX-11 treated HCC cells

**Conclusion:**
We found the underlying mechanism lactate-stimulated Kla on HCC metastasis. Histone H1.4 lactylation direct bond on the promoter region of MZF1 may assist its activation, facilitating the proliferation and metastasis of HCC cells (fig. 2J).

**Figure:**

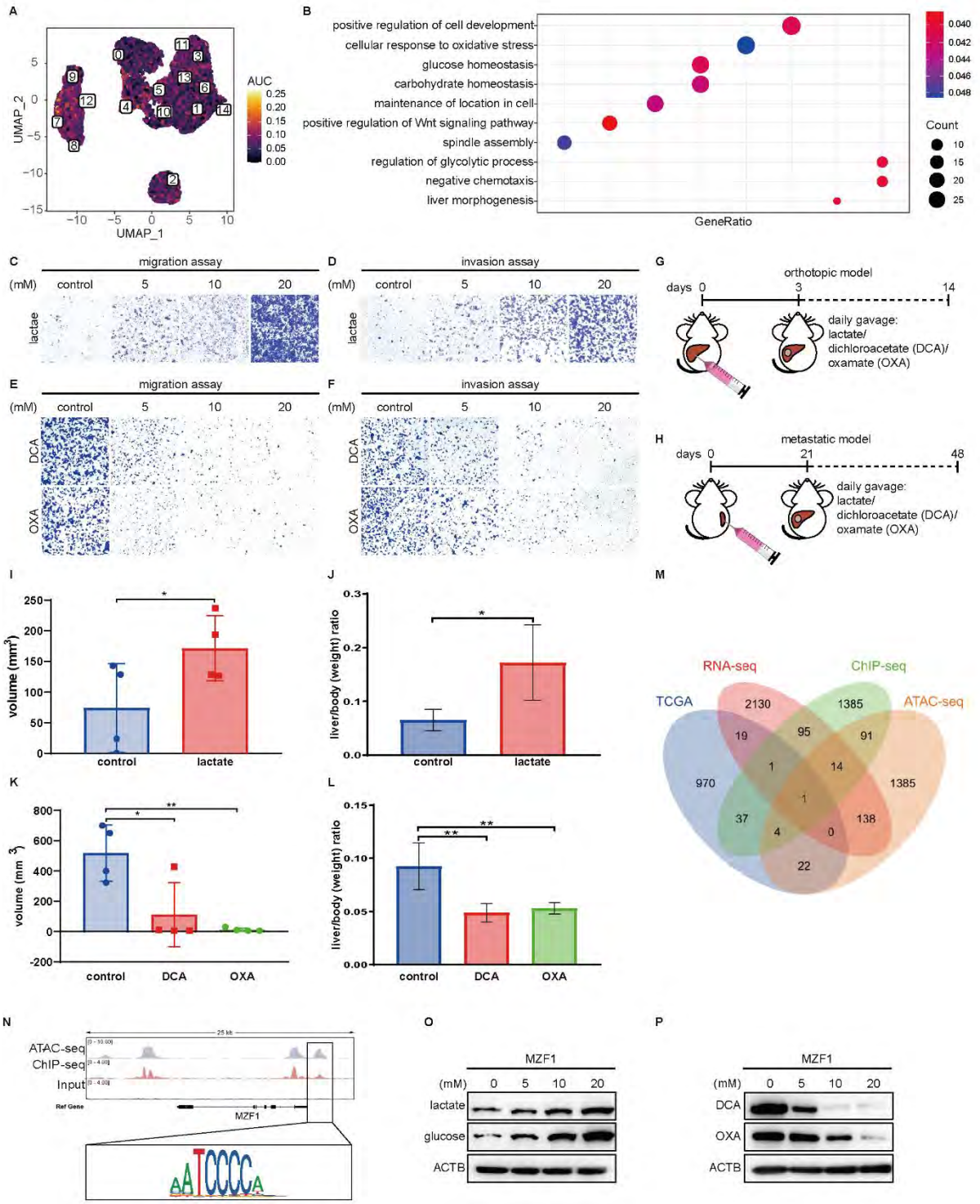
A
UMAP_2
UMAP_1
AUC
B
positive regulation of cell development
cellular response to oxidative stress
glucose homeostasis
carbohydrate homeostasis
maintenance of location in cell
positive regulation of Wnt signaling pathway
spindle assembly
regulation of glycolytic process
negative chemotaxis
liver morphogenesis
GeneRatio
Count
C
migration assay
(mM) control 5 10 20
lactae
D
invasion assay
E
DCA
OXA
F
G
orthotopic model
days 0 3 14
daily gavage:
lactate/
dichloroacetate (DCA)/
oxamate (OXA)
H
metastatic model
days 0 21 48
I
volume (mm3)
control
lactate
J
liver/body (weight) ratio
K
volume (mm 3)
control DCA OXA
L
M
RNA-seq
ChIP-seq
TCGA
ATAC-seq
2130
1385
95
19
91
1
14
970
1385
1
37
138
4
0
22
N
ATAC-seq
ChIP-seq
Input
Ref Gene
MZF1
O
MZF1
(mM) 0 5 10 20
lactate
glucose
ACTB
P
MZF1
(mM) 0 5 10 20
DCA
OXA
ACTB

**Figure. 1**

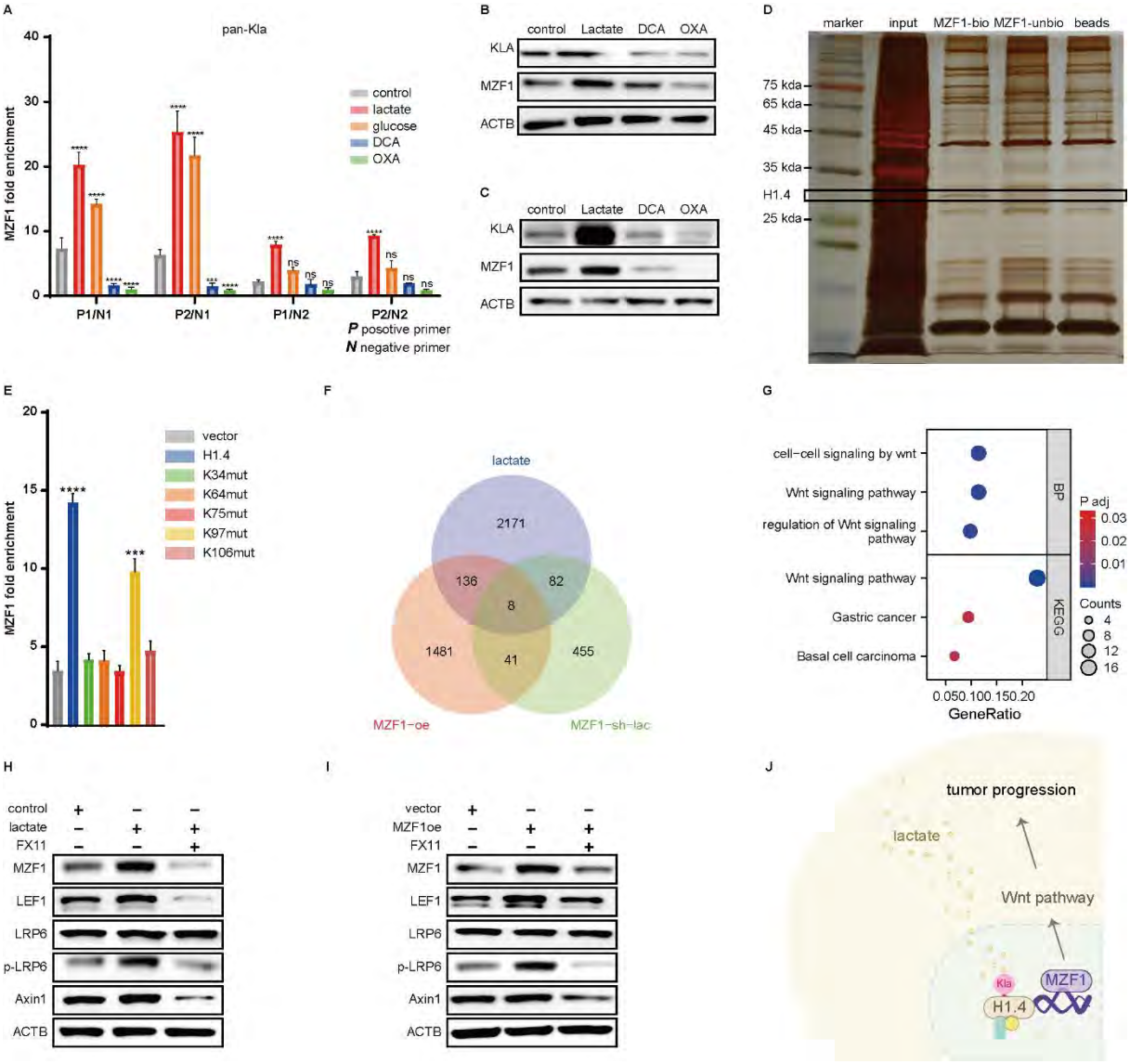
A
pan-Kla
MZF1 fold enrichment
control
lactate
glucose
DCA
OXA
P1/N1
P2/N1
P1/N2
P2/N2
P posotive primer
N negative primer
B
control Lactate DCA OXA
KLA
MZF1
ACTB
C
control Lactate DCA OXA
KLA
MZF1
ACTB
D
marker input MZF1-bio MZF1-unbio beads
75 kda
65 kda
45 kda
35 kda
H1.4
25 kda
E
MZF1 fold enrichment
vector
H1.4
K34mut
K64mut
K75mut
K97mut
K106mut
F
lactate
2171
136
82
8
1481
41
455
MZF1-oe
MZF1-sh-lac
G
cell-cell signaling by wnt
Wnt signaling pathway
regulation of Wnt signaling pathway
Wnt signaling pathway
Gastric cancer
Basal cell carcinoma
BP
KEGG
P adj
0.03
0.02
0.01
Counts
4
8
12
16
0.05 0.10 0.15 0.20
GeneRatio
H
control
lactate
FX11
MZF1
LEF1
LRP6
p-LRP6
Axin1
ACTB
I
vector
MZF1oe
FX11
MZF1
LEF1
LRP6
p-LRP6
Axin1
ACTB
J
tumor progression
lactate
Wnt pathway
Kla
MZF1
H1.4

**Figure. 2**

## OS-2
# Proteomic profiling of HCC reveals the involvement of energy metabolism in treatment response

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**Background and aims:** Hepatocellular carcinoma (HCC) is the fourth-most common cause of cancer-related mortality worldwide and is growing in incidence. Because only 50 to 70% of cancers are diagnosed late, its prognosis is extremely poor; that's why most advanced HCC are treated with regional or systemic palliative therapies. After 10 years of exclusive monotherapy with Sorafenib, currently, the anti-PDL1 (Atezolizumab) and anti-VEGFR (Bevacizumab) combination is the new recommended first-line treatment. However, the response rate to this combination therapy does not exceed 20%, according to studies and in case of failure, only 25% of patients can benefit from a second line of treatment. This is why it is crucial to make the right choice in first-line treatment to increase the level of response and survival.

**Method:** We have developed and patented a new proteomic profiling method that combines laser capture and high-resolution mass spectrometry for in-depth analysis of deregulated proteins in tumors. Our methodology is compatible with formalin-fixed and paraffin-embedded tissues (FFPET) as well as with very small amounts of material (diagnostic biopsies). Using a machine learning tool that we have recently developed (Dourthe et al, Hepatology 2021), we compare the tumor proteomic profiles of a liver tumor biopsy before treatment with those of a reference database composed of cases who have had an objective response or a progression under a given treatment. We validated the proteomics results by modifying protein expression and activation of biological pathways of interest in different HCC cell lines.

**Results:** Using a collection of 55 patients, we have identified a proteomic profile signature that discriminates patients with an objective response from patients with progression from their diagnostic biopsies for Atezolizumab/Bevacizumab and sorafenib. The proteomic signatures identified revealed the involvement of deregulation of energy metabolism enzymes as a predictor of response to treatment.

**Conclusion:** This study demonstrates the relevance of proteomic profiling of tumors to reveal new predictive signature of response to HCC treatments. We reveal here that energy metabolism conditions the response to treatments in HCC. This result paves the way for personalized patient care through proteomic profiling.

# OS-3
# Detecting early-stage hepatocellular carcinoma using novel methods: the ASAP model and circulating tumor DNA

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**Background and aims:** Liver cancer is a major cause of mortality worldwide, with an incidence of 854, 000 and 810, 000 deaths annually. Hepatocellular carcinoma (HCC) represents more than 90% of primary liver cancer, with an overall 5-year survival rate of 19.6%, but can be as low as 2.5% for advanced cases. In clinical practice, alpha-fetoprotein (AFP) and liver ultrasound are applied to diagnose HCC, but these methods lack sensitivity and more sensitive non-invasive biomarkers are urgently needed for early-stage detection of HCC.

**Method:** We conducted a multi-center study collecting over 2000 samples in 6 Latin American and 2 European countries. The performance of the GALAD score (AFP, AFP-L3, DCP, age, and sex) and an optimized version (ASAP score) was tested for pre-diagnostic and early-stage HCC detection. Also, DNA methylation markers (DMMs) *HOXA1*, *CLEC11A*, *AK055957*, and *TSPYL5* were tested in 146 liver tissues using quantitative methylation-specific PCR (qMSP), followed by validation and exploration of novel DMMs through methylated DNA sequencing (MeD-seq) on 44 liver tissues. DMMs explored by MeD-seq were further validated on circulating tumor DNA (ctDNA) from 18 HCC and 15 cirrhosis patients using qMSP.

**Results:** The GALAD score exhibited better performance than individual biomarkers to distinguish HCC from cirrhosis, with an AUC of 0.76 and 0.69 for HCC detection in Latin America and Europe, respectively. Logistic regression revealed that AFP-L3 contributed minimally to early-stage HCC detection. We modified the GALAD score by excluding AFP-L3, named ASAP score, with an AUC of 0.82 for early-stage HCC detection (64.6% sensitivity, 83.7% specificity). The ASAP score could predict HCC at 13 months pre-diagnosis. Besides tumor protein markers, we also assessed DNA methylation markers as biomarkers for HCC detection. Using qMSP and MeD-seq, we observed that methylation levels of DMMs are much higher in non-cirrhotic HCC tissues than control tissues. Novel DMMs identified by MeD-seq, such as *FOXD3* and *SPAG6*, showed good performance in discriminating cirrhotic HCC from cirrhotic controls. By qMSP on ctDNA isolated from plasma, we observed that methylation levels of DMMs are highly associated with late-stage HCC, but not early-stage, as compared to cirrhotic controls.

**Conclusion:** The optimized ASAP score is a good alternative for HCC prediction and detection. In blood, methylation levels of DMMs in ctDNA are highly correlated with late-stage HCC, but detection of early-stage HCC requires more sensitive detection methods.

# OS-4-YI
# Combining blood inflammatory scores predicts survival and reflects the tumor niche in intrahepatic cholangiocarcinoma

Flavio Milana[1 2], Michela Anna Polidoro[3], Cristiana Soldani[3], Simone Famularo[1 2], Barbara Franceschini[3], Ana Lleo[1 4], Matteo Donadon[5 6], Guido Torzilli[1 2]

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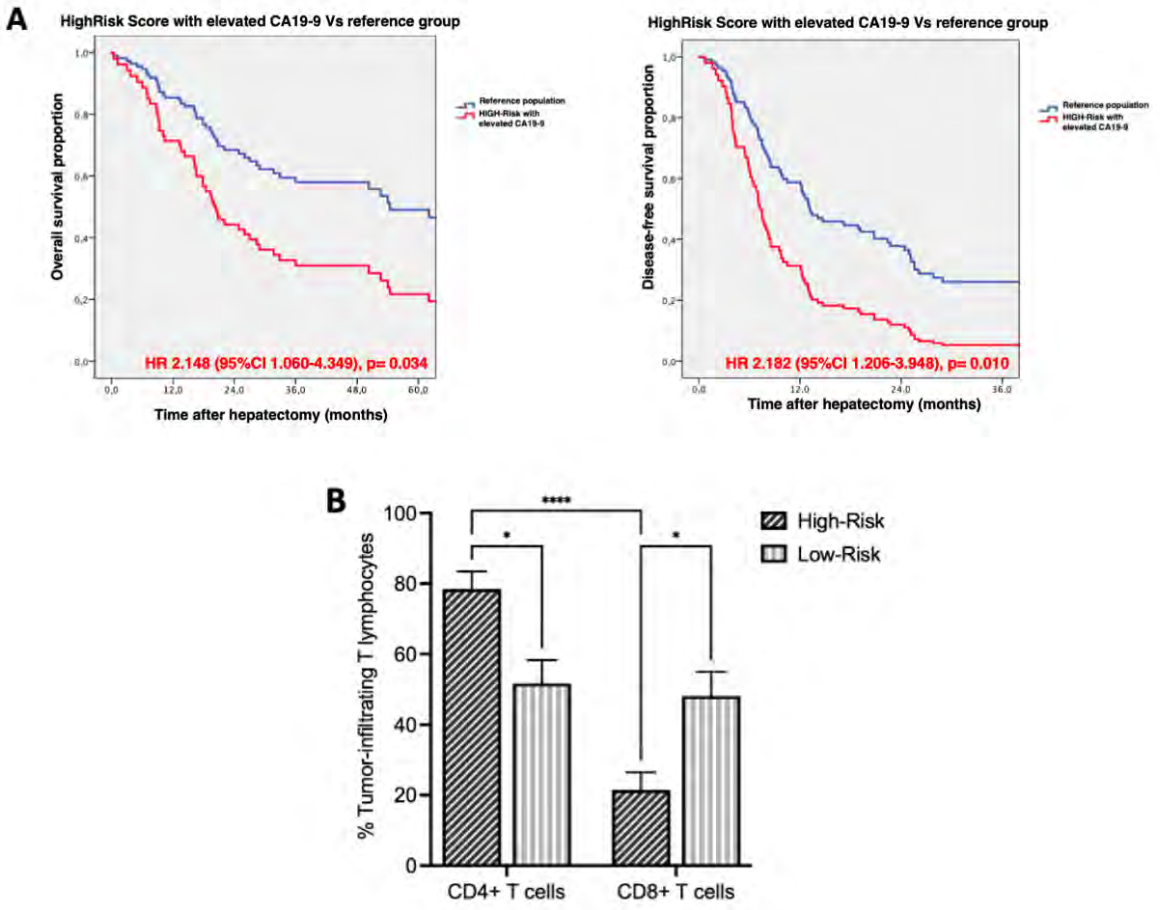
**Background and aims:** Systemic inflammation is relevant in intrahepatic cholangiocarcinoma (iCCA), but controversial results exist on the role of inflammatory indexes and their correlation with tumor microenvironment (TME). We aimed to explore the biological and prognostic values of these indexes.

**Method:** A cohort undergoing hepatectomy for iCCA between 2010-2021 was analyzed. The neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and clinic-pathological factors were recorded. Immune-cell subpopulations, isolated from surgical specimens, were analyzed by flow cytometry. NLR and LMR cut-offs were calculated by X-Tile software. Linear regression, Kaplan-Meier, and Cox regression analyses were conducted.

**Results:** A total of 101 iCCA patients were considered. NLR≥3.83 and LMR<2.28 correlated with worse survival. Patients were divided into groups: 67 (66.3%) in the low-risk and 34 (33.7%) in the high-risk (having at least one worse prognostic ratio). The 5-year overall survival (OS) was 49.8% and 18.9% for low- and high-risk groups, respectively (p = 0.003). At multivariate analysis, elevated CA19.9 (HR:1.969, 95%CI:1.072-3.618, p = 0.029) and high-risk group (HR:2.138, 95%CI:1.153-3.967, p = 0.016) were associated with worse OS. An elevated CA19.9 in the high-risk group gives 2.148 HR (95%CI: 1.060-4.349, p = 0.034) of mortality and 2.182 HR (95%CI: 1.206-3.948, p = 0.010) of disease recurrence (**Fig.1A**). According to flow cytometry analysis of 20 surgical specimens, a multivariate linear regression analysis highlighted that NLR was associated with tumor-derived NLR (p = 0.026) and LMR with tumor-infiltrating lymphocytes (p = 0.002) and tumor neutrophils (p = 0.016). In a subset of 5 high-risk vs 5 low-risk patients (**Fig.1B**), T-cell evaluation in the high-risk group showed a significantly higher prevalence of CD4+ T cells compared to CD8+ T cells (78.5% Vs 21.5%, p < 0.0001). Conversely, low-risk patients demonstrated comparable percentages of CD4+ and CD8+ T cells within the tumor niche (51.6% vs 48.7%, p = 0.998), with a noteworthy infiltration of CD8+ cells compared to the high-risk group (21.5% vs 48.7%, p = 0.037).

**Conclusion:** The combination of blood inflammatory indexes determined two survival-risk profiles. Adding CA19-9 identified patients at further increased recurrence risk. The strong correlation with the TME results suggests that the blood-derived clinical indexes, correlated with the patient's prognosis, reflect the iCCA microenvironment. The comparison among lymphocyte subpopulations suggests a potential link between distinct immune cell infiltration patterns and risk stratification based on inflammatory scores. These findings open the possibility of patient stratification with the chance to identify subgroups suitable for dedicated follow-up and targeted immuno-chemotherapy protocols.

**Figure:**


A
HighRisk Score with elevated CA19-9 Vs reference group
Overall survival proportion
Reference population
HIGH-Risk with elevated CA19-9
HR 2.148 (95%CI 1.060-4.349), p= 0.034
Time after hepatectomy (months)
HighRisk Score with elevated CA19-9 Vs reference group
Disease-free survival proportion
Reference population
HIGH-Risk with elevated CA19-9
HR 2.182 (95%CI 1.206-3.948), p= 0.010
Time after hepatectomy (months)
B
% Tumor-infiltrating T lymphocytes
High-Risk
Low-Risk
CD4+ T cells
CD8+ T cells

# OS-5
# Self-supervised learning to predict intrahepatic cholangiocarcinoma transcriptomic classes on routine histology

Beaufrère Aurélie[1], Tristan Lazard[2], Rémy Nicolle[3], Gwladys Lubuela[3], Jeremy Augustin[4], Miguel Albuquerque[1], Baptiste Pichon[3], Camille Pignolet[3], Victoria Priori[3], Nathalie Théou-Anton[5], Mickael Lesurtel[6], Mohamed Bouattour[7], Kévin Mondet[1], Jérôme Cros[1], Julien Calderaro[4], Thomas Walter[2], Valérie Paradis[1]

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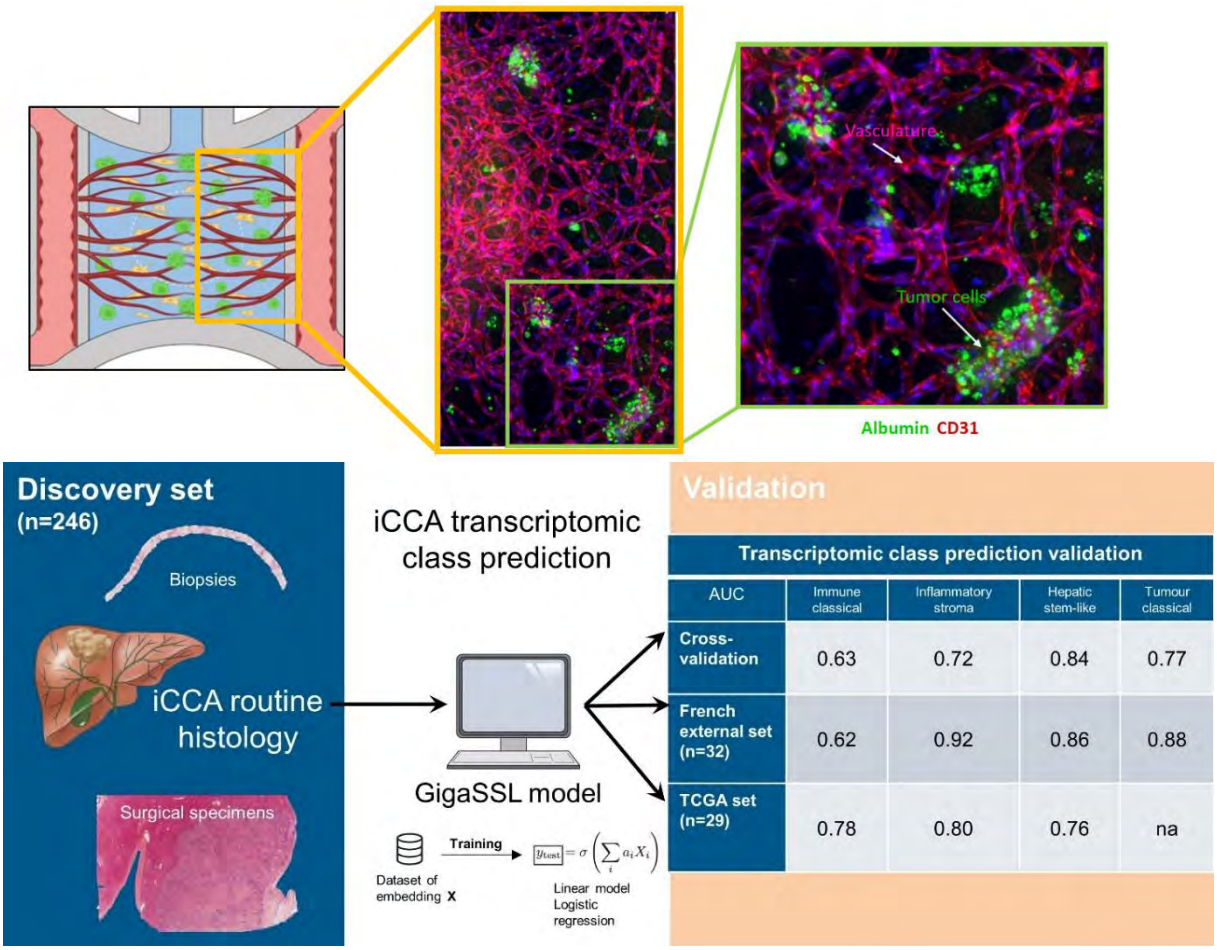
**Background and aims:** The transcriptomic classification of intrahepatic cholangiocarcinomas (iCCA) has been recently refined from two to five classes, associated with pathological features, targetable genetic alterations and survival. Despite its potential prognostic and therapeutic values, the transcriptomic classification is not routinely used in practice because of technical limitations, including insufficient tissue material or the cost of molecular analyses. Here, we assessed a self-supervised learning (SSL) model for predicting iCCA transcriptomic classes on whole-slide digital histological images (WSIs).

**Method:** The transcriptomic classification of intrahepatic cholangiocarcinomas (iCCA) has been recently refined from two to five classes, associated with pathological features, targetable genetic alterations and survival. Despite its potential prognostic and therapeutic values, the transcriptomic classification is not routinely used in practice because of technical limitations, including insufficient tissue material or the cost of molecular analyses. Here, we assessed a self-supervised learning (SSL) model for predicting iCCA transcriptomic classes on whole-slide digital histological images (WSIs).

**Results:** The most frequent transcriptomic class was the hepatic stem-like class [37% (90/246) in the discovery set]. Our model showed good to very good performance in predicting the four most frequent transcriptomic classes in the discovery set (area under the curve [AUC]: 0.63-0.84), especially for the hepatic stem-like class (AUC 0.84). The model performed equally well in predicting these transcriptomic classes in the two validation sets, with AUCs ranging from 0.76 to 0.80 in the TCGA set and 0.62 to 0.92 in the French external set.

**Conclusion:** We developed and validated a SSL-based model for predicting iCCA transcriptomic classes on routine histological slides of biopsy and surgical samples, which may positively impact iCCA management by predicting patient's prognosis and guiding the treatment strategy.

**Figure:**


Vasculature
Tumor cells
Albumin CD31
Discovery set
(n=246)
Biopsies
iCCA routine histology
Surgical specimens
iCCA transcriptomic class prediction
GigaSSL model
Training
Dataset of embedding X
Linear model Logistic regression
Validation
Transcriptomic class prediction validation
AUC
Immune classical
Inflammatory stroma
Hepatic stem-like
Tumour classical
Cross-validation
0.63
0.72
0.84
0.77
French external set (n=32)
0.62
0.92
0.86
0.88
TCGA set (n=29)
0.78
0.80
0.76
na

# OS-6
# mRECIST outcomes in EMERALD-1: a Phase 3, randomized, placebo-controlled study of transarterial chemoembolization plus durvalumab with/without bevacizumab in participants with embolization-eligible hepatocellular carcinoma

Bruno Sangro[1], Masatoshi Kudo[2], Joseph Erinjeri[3], Shukui Qin[4], Zhenggang Ren[5], Stephen Chan[6], Yasuaki Arai[7], Jeong Heo[8], Ahn Mai[9], Jose Escobar[10], Yamil Alonso Lopez Chuken[11], Jung-Hwan Yoon[12], Won Young Tak[13], Tanita Suttichaimongkol[14], Mohamed Bouattour[15], Shi-Ming Lin[16], Magdalena Żotkiewicz[17], Sajid Ali[18], Gordon Cohen[19], Riccardo Lencioni[20]

*[1]Clinica Universidad de Navarra, Liver Unit and HPB Oncology Area, Pamplona, Spain*, *[2]Kindai University, Department of Gastroenterology and Hepatology, Higashiosaka, Japan*, *[3]Memorial Sloan Kettering Cancer Center New York, Interventional Radiology Service, New York, United States*, *[4]Jinling Hospital, Cancer Center of Nanjing, Nanjing, China*, *[5]Zhongshan Hospital Fudan University, Department of Hepatic Oncology, Liver Cancer Institute, China*, *[6]The Chinese University of Hong Kong (CUHK), Department of Clinical Oncology, Sir Yue-Kong Pao Center for Cancer, Hong Kong, Hong Kong*, *[7]National Cancer Center Hospital, Department of Diagnostic Radiology, Chuo City, Japan*, *[8]Pusan National University Hospital, Department of Internal Medicine, College of Medicine, Pusan National University and Biomedical Research Institute, Busan, Korea, Rep. of South*, *[9]Nhan dan Gia Dinh Hospital, 9General Surgery Department, Viet Nam*, *[10]Hospital San Lucas, Chiapas, Mexico*, *[11]I Can Oncology Center, Monterrey, Mexico*, *[12]Seoul National University College of Medicine, Seoul, Korea, Rep. of South*, *[13]Kyungpook National University (KNU), Daegu, Korea, Rep. of South*, *[14]Khon Kaen University, Division of Gastroenterology and Hepatology, Department of Medicine, Faculty of Medicine, Thailand*, *[15]Hospital Beaujon AP-HP, Medical Oncology, Paris, France*, *[16]Linkou Chang Gung Memorial Hospital, Department of Internal Medicine, Taiwan*, *[17]Oncology Biometrics, Late Oncology Statistics, AstraZeneca, Warszawa, Poland*, *[18]Late Development Oncology, AstraZeneca, United Kingdom*, *[19]AstraZeneca, Global Medicines Development, Gaithersburg, United States*, *[20]University of Pisa, Department of Diagnostic and Interventional Radiology, Pisa, Italy*

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**Background and aims:** For >20 years, transarterial chemoembolization (TACE) has been a standard of care for embolization-eligible unresectable hepatocellular carcinoma (uHCC); however, most people with uHCC treated with TACE progress within 1 year. The primary end point of EMERALD-1 (NCT03778957) was positive, with a statistically significant improvement in progression-free survival (PFS) assessed by blinded independent central review (BICR) per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 with durvalumab (D) + bevacizumab (B) + TACE vs TACE (Table). Safety was consistent with the profiles of D, B, and TACE in uHCC. In addition to RECIST v1.1, efficacy of the regimens was assessed using modified RECIST (mRECIST).

**Method:** Participants (pts) with embolization-eligible uHCC, Child-Pugh A to B7 liver function, Eastern Cooperative Oncology Group performance status 0-1, and no evidence of extrahepatic disease were randomized 1:1:1 to the D+B+TACE, D+TACE, or TACE arms. TACE was cTACE or DEB-TACE (investigator choice). Pts received D (1500 mg) or placebo for D (Q4W) plus TACE. After completion of last TACE, pts received D (1120 mg) plus placebo for B, D (1120 mg) plus B (15 mg/kg), or placebos for D and B (Q3W). PFS was assessed by BICR and the investigator per mRECIST as a secondary end point.

**Results:** PFS improved with D+B+TACE vs TACE as assessed by both BICR per mRECIST and the investigator per mRECIST (Table). The estimation of effect was consistent with that of the primary end point, which showed a statistically significant improvement in PFS with D+B+TACE vs TACE, as assessed by BICR per RECIST v1.1 ($p = 0.032$, significance threshold 0.0435; Table). D+TACE vs TACE showed similar PFS results as assessed per mRECIST and RECIST v1.1 (Table).

**Conclusion:** D+B+TACE is the first immune checkpoint inhibitor-based regimen in a global Phase 3 trial to show statistically significant improvement in PFS vs TACE in pts with embolization-eligible uHCC, as assessed by BICR per RECIST v1.1, with consistent PFS results, as assessed per mRECIST. These results further support the potential for D+B+TACE to set a new standard of care in uHCC

**Figure:**

| | **BICR RECIST v1.1 (primary end point)** | | | **BICR mRECIST** | | | **Investigator mRECIST** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **D+TACE (N = 207)** | **D+B+TACE (N = 204)** | **TACE (N = 205)** | **D+TACE (N = 207)** | **D+B+TACE (N = 204)** | **TACE (N = 205)** | **D+TACE (N = 207)** | **D+B+TACE (N = 204)** | **TACE (N = 205)** |
| Median PFS, months (95% CI) | 10.0 (9.0-12.7) | 15.0 (11.1-18.9) | 8.2 (6.9-11.1) | 9.0 (7.0-10.9) | 14.2 (11.1-17.4) | 8.2 (6.9-9.5) | 7.0 (6.7-9.0) | 13.2 (9.4-16.4) | 7.5 (7.0-9.1) |
| PFS HR vs TACE (95% CI) | 0.94 (0.75-1.19), p = 0.638 | 0.77 (0.61-0.98), p = 0.032 | | 0.99 (0.79-1.25) | 0.75 (0.60-0.95) | | 1.03 (0.84-1.28) | 0.69 (0.55-0.86) | |
| B, bevacizumab; BICR, blinded independent central review; D, durvalumab; HR, hazard ratio; (m)RECIST, (modified) Response Evaluation Criteria in Solid Tumors; PFS, progression-free survival; TACE, transarterial chemoembolization. | | | | | | | | | |

# OS-7
# Recapitulating hepatocellular carcinoma's complex biology and diverse treatment response in a patient-derived microfluidic model

Orsola Mocellin[1], Manuel Caro Torregrosa[1], Abbie Robbinson[1], Stephane Treillard[1], Thomas Olivier[1], Chee Ng[1], Jeroen Heijmans[1], Arthur Stok[1], Gilles van Tienderen[2], Monique M.A. Verstegen[2], Flavio Bonanini[1], Sebastiaan Trietsch[1], Henriette Lanz[1], Paul Vulto[1], Jos Joore[1], Karla Quieroz[1]
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**Background and aims:** Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and is closely associated with progressive stages of liver diseases. Its transformation, survival, progression and metastasis have been associated with regulation mechanisms orchestrated by the tumor stroma. Patient differences in tumor composition and cellular signaling are reflected in the diversity of therapy response. Hence, comprehensive in vitro models that capture patient-specific tumor and stromal components are necessary to elucidate and evaluate anticancer therapy.

**Method:** Patient-derived dissociated HCC cells from eight different patients were combined with tumor-derived fibroblasts and endothelial cells within a microfluidic setup comprising 64 parallel chips in a microtiter plate format. Cultures were challenged with an individual or combinatorial treatment from a panel of tool compounds for 72 hours in an automated setup. We used immunofluorescence and high content confocal imaging to assess the structural and biological interaction between cancer cells and the associated stroma. Viability assessment, artificial intelligence-based vascular morphology characterization and multiplexed chemokine/cytokine release measurements were used to elucidate patient and drug-dependent effects of single or combinatorial treatments.

**Results:** Cellular interaction within the microfluidic platform led to a vascularized tumor construct, with hepatocellular carcinoma aggregates being enveloped and traversed by a lumenized and interconnected vascular plexus, in association with tumor-derived fibroblasts. Compound treatment led to profound differences across the tested parameters, indicating different anticancer effects in conjunction with the biological diversity of the patient material. Finally, morphological assessment revealed distinct compound-induced effects on the tumor's vascular organization

**Conclusion:** We present a vascularized patient-derived HCC model that includes relevant cellular players of the tumor microenvironment found in vivo. These co-cultures are highly suitable for studying specific cell types as well as patient-specific responses. We envision that this system has the potential to provide a platform for understanding the interplay between different cell types present in hepatocellular carcinomas, with sufficient scalability ease of use for industrial and clinical implementation.

**Figure:** Cultures show organized vascular networks and tumor aggregates directly interacting with the vasculature.

## OS-8-YI

# The survival impact of the addition of durvalumab to cisplatin/gemcitabine in advanced biliary tract cancer: a real-world, retrospective, multicentric study

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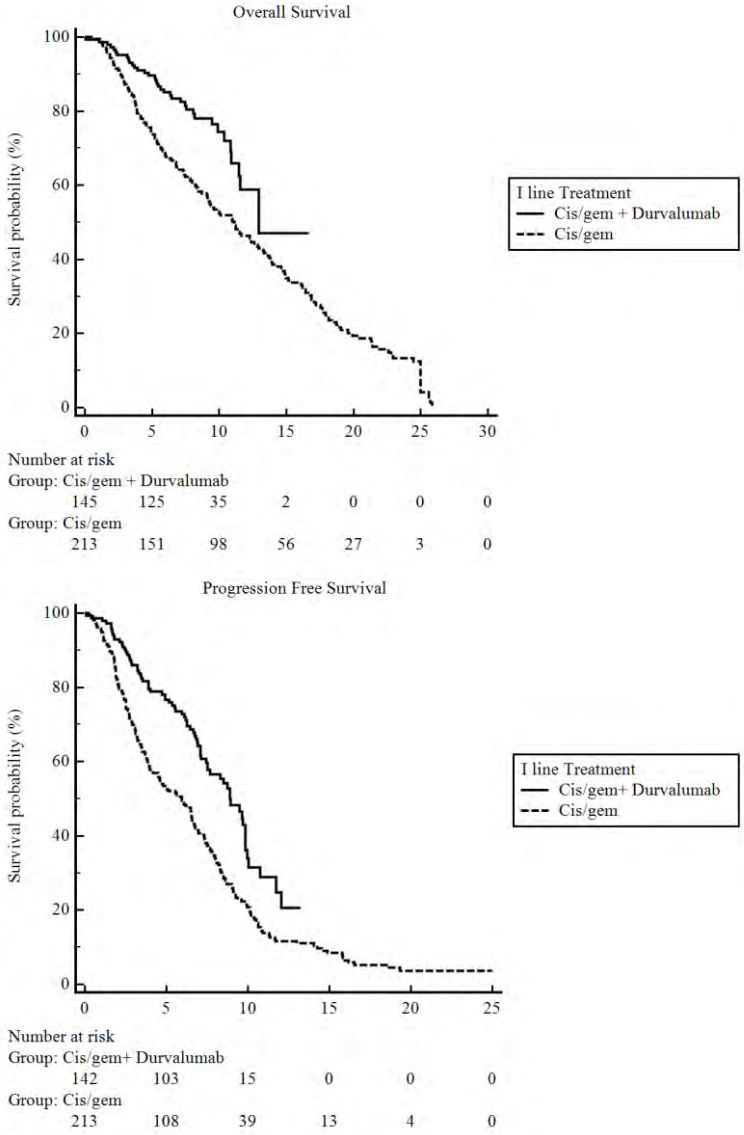
**Background and aims:** The present study investigated for the first time the survival impact resulted from the addition of durvalumab to cisplatin/gemcitabine in a real-world cohort of biliary tract cancer (BTC) patients.

**Method:** The analyzed population included patients with unresectable, locally advanced, or metastatic BTC treated with durvalumab in combination with cisplatin/gemcitabine or cisplatin/gemcitabine alone. The impact of the addition of durvalumab to chemotherapy in terms of both overall survival (OS) and progression free survival (PFS) was investigated with uni- and multivariate analysis.

**Results:** 358 patients were included in the analysis: 213 received cisplatin/gemcitabine alone, 145 received cisplatin/gemcitabine plus durvalumab. The median OS and PFS were 11.2 Vs 12.9 months (HR 1.8, 95% CI 1.3-2.5, p = 0.0005) and 6.0 Vs 8.9 months (HR 1.9, 95% CI 1.4-2.3, p <0.0001) in patients who received cisplatin/gemcitabine alone compared to those who received cisplatin/gemcitabine plus durvalumab, respectively. The multivariate analysis confirmed that the addition of durvalumab to cisplatin/gemcitabine is a independent prognostic factor for both OS and PFS. NLR>3 and ECOG PS>0 resulted to be independent prognostic factors and predictive factors of response to cisplatin/gemcitabine plus durvalumab.

**Conclusion:** The addition of durvalumab to cisplatin/gemcitabine has been confirmed to confer a survival benefit in terms of both OS and PFS in a real-world setting of advanced BTC patients.

**Figure:**


Overall Survival
Survival probability (%)
100
80
60
40
20
0
0 5 10 15 20 25 30
I line Treatment
Cis/gem + Durvalumab
Cis/gem
Number at risk
Group: Cis/gem + Durvalumab
145 125 35 2 0 0 0
Group: Cis/gem
213 151 98 56 27 3 0
Progression Free Survival
Survival probability (%)
100
80
60
40
20
0
0 5 10 15 20 25
I line Treatment
Cis/gem+ Durvalumab
Cis/gem
Number at risk
Group: Cis/gem+ Durvalumab
142 103 15 0 0 0
Group: Cis/gem
213 108 39 13 4 0

## OS-9-YI

# Immunosuppressive contribution of tumor-infiltrating B cell subsets in human intrahepatic cholangiocarcinoma and their role in immunotherapy response

Giulia Milardi[1], Barbara Franceschini[1], Guido Costa[2], Cristiana Soldani[1], Paolo Uva[3], Davide Cangelosi[3], roberta carriero[4], Giulio Lodetti Zangrandi[1], Ines Malenica[5], Marco Erreni[6], Rita Balsano[7], Tiziana Pressiani[7], Barbara Cassani[8 9], Guido Torzilli[2 10], Lorenza Rimassa[7 10], Ana Lleo[10 11]

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**Background and aims:** intrahepatic cholangiocarcinoma (iCCA) is a heterogeneous biliary tract cancer whose incidence rate increased over the past decades. Due to the aggressive evolution of the disease, there is an urgent need for diagnostic and therapeutic alternatives. The immune infiltrate is a key component of the tumor microenvironment (TME), but remains poorly characterized, limiting the development of successful immunotherapies. Aspects related to T cells are undergoing extensive studies, while the effect exerted by B lymphocytes in iCCA development and progression is still controversial.

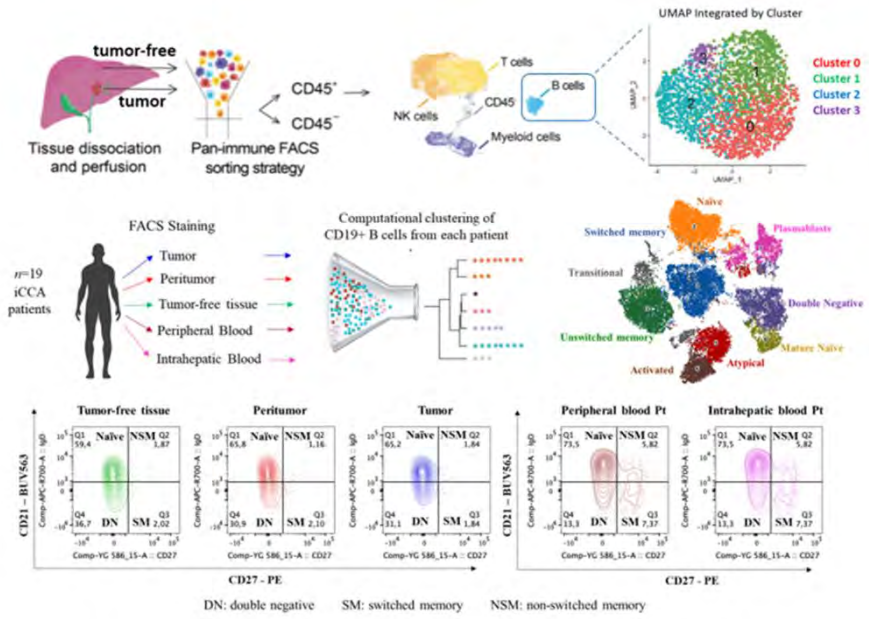
**Method:** we characterized the B-cell compartment of tumor, tumor-free tissue and circulating counterpart of iCCA patients, using single-cell RNA-sequencing technologies. We further carried out gene expression analysis and cellular assays to define B cell properties, investigating whether and how liver TME impacts B cell biology.

**Results:** results from single-cell RNA-sequencing of CD20+ cells in six iCCA patients identified four main subclusters and revealed a down-regulation of B cell activation/inflammatory genes in tumor compared to non-malignant tissue; suggesting an immunosuppressive condition of B cells in the TME. Multicolor flow cytometry analysis of B lymphocytes isolated from iCCA patients (n = 19) highlighted a higher frequency of naïve B cells with respect to memory B in the tissue samples. Gene expression analysis and multiplex beads arrays showed higher expression levels and production of immunoregulatory cytokines within the tumor compared to non-tumoral tissue. A lower level of immunoglobulins was also detected in iCCA plasma samples. This may be caused by tumor and stromal cells that affect B cell skewing and activation state. Immunohistochemical analyses highlighted that B cells, when infiltrate the tissues, create cellular aggregates similar to tertiary lymphoid structures (TLS). TLS density outside the tumor area positively correlated with better disease-free survival.

Circulating B cells from iCCA patients (n = 19) treated with chemo-immunotherapy (gem-cis plus durvalumab) underwent phenotypic and functional variations. Responder patients showed a higher frequency of transitional and naïve B cells, more activated than the non-responder group, where we found an increase of memory B and plasmablasts.

**Conclusion:** results sustain the heterogeneity of the B cell population within the TME and peripheral blood of iCCA patients, with a potential contribution to immunosuppressive function in the tumor burden. Findings from these cohorts also provide important insights into the role of B cells in response to chemo-immunotherapy. A deeper analysis of B cell subsets and crosstalk with other cells of the iCCA milieu will be exploitable for improving the therapy's effectiveness and developing new ones.

**Figure:**




The present work was partially funded by the Associazione Italiana per la Ricerca sul Cancro (IG AIRC 2019 – ID 23408 to A. Lleo)

## OS-10
# Treatment of hepatocellular carcinoma with Y-90 glass microspheres: results from the proactif study

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*[1]Hôpital Henri Mondor Créteil*, *[2]Centre Eugène Marquis Rennes*, *[3]CHU Nantes*, *[4]CHU Bordeaux*, *[5]Hôpital de la Timone*, *[6]Hôpital Paul Brousse*, *[7]CHU Grenoble*, *[8]Institut Paoli-Calmettes*, *[9]Centre Henri Becquerel Rouen*, *[10]CHU Dijon*, *[11]Hopital Fernand Widal*

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**"The abstract has been removed at the request of the authors."**

# OS-11
# Nurse led follow-up for patients with advanced hepatocellular carcinoma on systemic anti cancer therapy

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**Background and aims:** Improvements in overall survival along with reduced toxicity (Finn et al 2020), increasing number of patients having systemic therapy for Hepatocellular Carcinoma (HCC) and living longer with advanced disease. As patients are living longer, there is increased nursing needs from patients and those around them-from living with their cancer, treatment and liver related side effects alongside the psychological impact of this on patients.Within a referral centre has seen an increase in new diagnoses, increase in referrals and increase in treatment duration. The local audit led us to explore the role of nurse follow-up and support for patients; safety data and patient experience of nurse led follow-up alongside looking at skills and resources needed to support.

**Method:** As part of the service audit assessing clinic activity, patient consultation time and toxicity management and prescribing activity. This showed patients established on treatment their consultation was 12 minutes and risk and safety profile showed low incidence of recording of toxicity at 12% and these represented grades 1 and 2 toxicity. Patient focus group showed patients feel safe under the nurses' care and see them as working as part of a wider team. Feedback included:
'the nurse is easy to talk to'
'they understand my needs'
'they are knowledgeable'
Skills including history taking, management of liver disease, toxicity management and qualifications including advanced assessment skills and non-medical prescribing including SACT prescribing were agreed. As part of the clinic, the nurse was able to use their knowledge of liver disease alongside treatment related side effects alongside providing holistic management of patient needs.

**Results:** 30 patients were reviewed in the nurse led SACT clinic. No reported delays to treatment and supportive medication prescriptions were 14% (in line with medical clinic review).
The nurse led clinic was also able to incorporate quality of life and support as part of the consultation. Initial feedback from the patient survey was positive with no negative feedback.
With 30 patients transitioned to nurse led follow-up, consultant led new patient capacity was increased by 50%.
**Conclusion:** We were able to show positive safety data from nurse led follow-up with experienced Hepatocellular Carcinoma Clinical Nurse specialist delivering pre-treatment consultations and positive patient feedback. There was no increase in delays to treatment within non-medical clinic.
Structured infrastructure and investment in staffing and educational development is important to build this aspect of patient care alongside knowledge and skills of liver disease management and SACT toxicity. Consultant oversight and support is pivotal to success and clear escalation policy.
Further patient experience and longer-term data is awaited.

# POSTER ABSTRACT PRESENTATIONS

# Basic Science

## PO1-08

# Pirfenidone promotes tumor cell elimination via increased infiltration of cytotoxic cells and by limiting fibrosis and responses of regulatory T cells in an experimental hepatocellular carcinoma model

Scarlet Arceo-Orozco[1-2-3], Fernando Caloca Camarena[4], Hugo Christian Monroy-Ramirez[4], Marina Galicia Moreno[4], juan armendariz-borunda[4-5]

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**Background and aims:** Hepatocellular carcinoma (HCC) is the most common liver neoplasm worldwide, and one of the main causes of cancer related death. Projections are not encouraging, as it is estimated that by 2030, this health problem will increase. Beyond representing a diagnostic-therapeutic challenge, HCC is considered a health problem due to its high rate of relapses and recurrences. Pro-inflammatory, pro-oxidative and pro-fibrogenic processes are key in the development and spreading of tumors. Pirfenidone (PFD) has demonstrated hepatoprotective properties during hepatocarcinogenesis; however, its effects on established HCC are unknown. Our aim was to evaluate the effects of PFD on the tumor microenvironment, and inflammatory processes in an experimental hepatocarcinoma model.

**Method:** Fischer-344 rats (n = 18) were divided into three groups: CTL (control), HCC (injury group with Diethylnitrosamine (DEN;50 mg/kg/i.p), and 2-Acetylaminofluorene (2AAF;25mg/kg/p.o.) weekly both treatments, HCC/PFD: injury + PFD 300 mg/kg/p.o. daily. Histological and molecular analyses were performed to evaluate malignant, inflammatory, and fibrotic patterns.

**Results:** The HCC/PFD group exhibits a lower number, size, and protrusion of neoplastic nodules compared to the HCC group. Additionally, fibrosis development was slowed down in the HCC/PDF group, limiting the formation of thick fibrotic bridges, and correlating with a decrease in alpha-SMA expression. Damage patterns were also limited, and the percentage of hepatocytes positive for Glypican-3 and Ki-67 decreased, as opposed to increased p53 and caspase 3 protein levels in the HCC/PFD group. Expression and co-localization of cells positive for CD45 and CD161between tumor niches were promoted by PFD, meanwhile the migration of FOXP3-positive cells was restricted in the HCC/PFD group versus the HCC group. Moreover, PFD administration significantly modified the local secretome as expression of interleukins IL-10, IL-17 and IL-1beta expression were decreased.

**Conclusion:** PFD treatment reduces HCC development, slows down macro- and microscopic malignant and fibrotic patterns, decreases hepatic stellate cell activation, and modulates the tumor microenvironment, limiting neoplastic progression. Therefore, this work suggests that PFD treatment could represent an improvement in quality of life and survival of patients with advanced-stage HCC.

**Figure:**

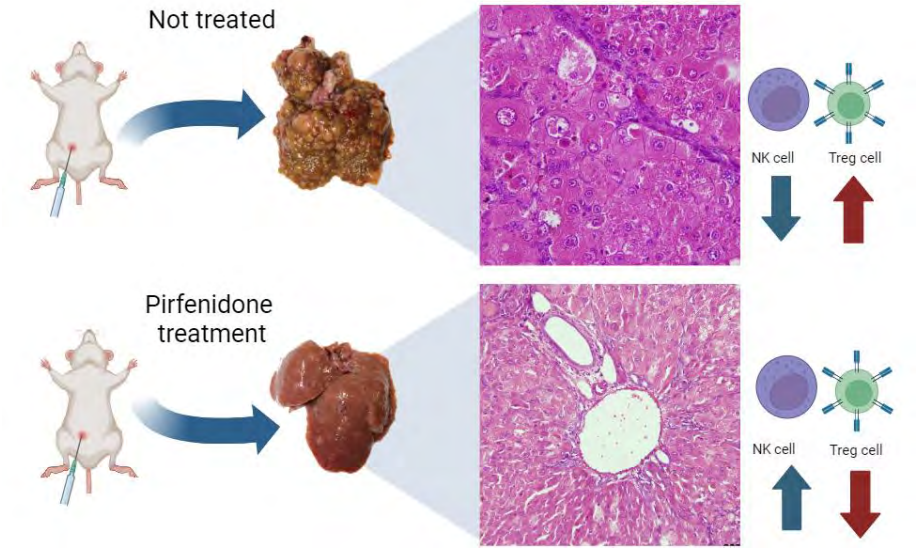


Figure:The administration of PFD facilitated cytotoxic cell responses over regulatory T cells.

## PO1-09-YI
# Usefulness of inhibiting the drug-export pump MRP3 for sensitizing cholangiocarcinoma to chemotherapy

Maitane Asensio[1 2], Oscar Briz[1 2], Elisa Herraez[1 2], Ricardo A. Espinosa-Escudero[1], Laura Perez-Silva[1], Marta Romero[1 2], Alvaro Temprano[1], maria reviejo[1 2], Nazaret Hortelano-Hernandez[1], Rebeca P. marijuan[1], Ana Peleteiro Vigil[1], Oliver Poetz[3 4], Rocio IR Macias[1 2], Onat kadioglu[5], Thomas Efferth[5], Elisa Lozano[1 2], Jose Marin[1 2]

*[1]1) Experimental Hepatology and Drug Targeting (HEVEPHARM), IBSAL, University of Salamanca, Spain, [2]2) National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Carlos III National Health Institute, Madrid., Spain, [3]Signatope GmbH, Reutlingen, Germany, [4]4) Natural and Medical Sciences Institute at the University of Tubingen (NMI), Reutlingen, Germany, [5]5) Department of Pharmaceutical Biology, Institute of Pharmaceutical and Biomedical Sciences, Johannes Gutenberg University, Mainz, Germany*

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**Background and aims:** Active export of antitumor drugs through ATP-binding cassette (ABC) proteins hinders the pharmacological response of many cancers. The relevance of MRP3 (gene ABCC3) as a drug transporter in cholangiocarcinoma (CCA) is still unknown. Here, we have evaluated the usefulness of MRP3 as a target to reverse drug resistance in CCA.

**Method:** Expression levels were analyzed in silico using data from CCA available at the TCGA database and determined by RT-QPCR in intrahepatic (iCCA) and extrahepatic (eCCA) CCA samples and paired adjacent liver tissue (Salamanca cohort). Protein expression and subcellular location were assayed by immunoaffinity LC-MS/MS+WB and IHC, respectively. Different monoclonal cell lines with overexpression or silencing of MRP3 were developed by transduction with lentiviral vectors. Cell viability was determined by MTT test. Interaction between MRP3 and ~40.000 natural and semisynthetic compounds found in ZINC database was predicted by docking studies. Tyrosine kinase inhibitors (TKI) were also screened as potential MRP inhibitors. The ability of the selected compounds to inhibit MRP3 was validated by flow cytometry and cell viability assays. SynergyFinder online tool was used to calculate the degree of synergistic-additive-antagonistic effects of drug combinations.

**Results:** MRP3 was the most expressed ABCC pump both in iCCA and eCCA samples. CCA-derived cells (EGI-1 and TFK-1) showed high ABCC3 levels and strong staining consistent with MRP3 expression at the plasma membrane. Overexpression of ABCC3 resulted in a significantly lower cytostatic effect of etoposide (a known MRP3 substrate), platinum-based drugs, SN-38, 5-FU, mitoxantrone, and cabozantinib as compared with Control cells. Besides, silencing ABCC3 in EGI-1 cells using shRNAs resulted in enhanced sensitivity to etoposide, cisplatin, SN-38, and mitoxantrone. In silico screening of natural compounds identified 8 potential MRP3 inhibitors with high ligand binding energy. The study by flow cytometry-based transport assays of the inhibitory activity of these potential chemosensitizers and 20 TKIs revealed that EM-114, EM-188, and sorafenib reduced the ability of MRP3 to transport carboxyfluorescein, a well-known MRP substrate, in different cellular models that express MRP3. However, only sorafenib and MK571 (a pan-MRP inhibitor) showed the ability to sensitize CCA cell lines to agents presumably transported by MRP3, such as etoposide, SN38, and cisplatin. Thus, combining etoposide or cisplatin with sorafenib synergistically reduced the viability of CCA cells.

**Conclusion:** The drug export pump MRP3, which is highly expressed in CCA, may determine the response of this cancer to several antitumor drugs, like cisplatin and SN-38. Moreover, pharmacological inhibition of MRP3 with sorafenib can be a useful synergistic strategy to enhance the response of CCA to chemotherapy.

# PO1-11
# Differential deregulation of the metabolome during liver cancer progression and regression

yuhai hu[1], Mari Teuter[2], Frank Bengel[2], Jens Bankstahl[2], Michael Ott[1], Asha Balakrishnan[1]

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**Background and aims:** Changes in metabolism is a defining characteristic of many cancers, including hepatocellular carcinoma (HCC), the most common type of liver cancer. It is therefore imperative to identify alterations in the metabolome as liver tumors develop, regress and recur. This in turn would help identify novel and more effective targets for HCC treatment. **Aims:** (1). Study the tumor metabolome during tumor development, regression and recurrence. (2). Analyze the therapeutic effects of metabolism-associated microRNAs on HCC, *in vivo*.

**Method:** We performed global metabolomics on liver tissues from transgenic oncogene-driven conditional model of HCC, which were at different stages of liver tumor development, regression and recurrence. We also studied metabolic flux to characterize these different stages using serial multi-tracer PET/CT on two independent transgenic oncogene-driven mouse models of HCC. Data was compared between the stages of tumor development and regression, as well as between the different oncogene-driven models during HCC development. *In vitro* and *in vivo* modulation of specific microRNAs was done to analyze their therapeutic efficacy in HCC attenuation via their regulation of metabolic reprogramming.

**Results:** We found that different stages of HCC development, regression and recurrence show distinct metabolic profiles. Our serial PET/CT analyses additionally showed that in addition to these different stages, metabolic changes were also distinct between the different oncogene-dependent mouse models. Further, therapeutic modulation of specific metabolism-associated microRNAs, significantly attenuated HCC development and prolonged survival in these tumor-bearing mouse models.

**Conclusion:** Changes in metabolism occur very early in liver cancer development, even before any obvious tumor develops. The metabolome significantly, distinctly and dynamically reprograms during tumor development, regression and even as tumors recur. Studying these changes would help identify novel biomarkers as well as targets for therapeutic intervention in HCC. Therapeutic modulation of non-coding RNAs that regulate specific targets in metabolic pathways, indicate a promising approach for HCC treatment.

## PO1-12

# High-dimensional spatial profiling of the hepatocellular carcinoma tumor microenvironment reveals spatial immune types informing immune checkpoint inhibitor therapy response

Henrike Salie[1], Lara Wischer[1], Patricia Otto-Morra[1], Juergen Beck[1], Antonio D'Alessio[2], Olaf Neumann[3], Albrecht Stenzinger[3], Andreas Blaumeiser[4], Melanie Boerries[4], Robert Thimme[1], David J. Pinato[2], Thomas Longerich[3], Bertram Bengsch[1 5 6]

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**Background and aims:** Hepatocellular carcinoma (HCC) is a heterogeneous entity with distinct subtypes described based on their molecular profiles, including immune subclasses. However, the utility of these classifications for the prediction of immunotherapy outcomes is limited. We hypothesized that the spatial organization of the immune response in the tumor microenvironment (TME) is likely to influence response and survival of HCC patients under immune checkpoint inhibitor (ICI) therapy. Thus, we set out to characterize the immune architecture of the HCC TME on a spatially resolved, high-dimensional single-cell level using highly multiplexed imaging mass cytometry (IMC) and to develop a classification that correlates with immunotherapy outcome.

**Method:** Highly multiplexed IMC analysis was applied to FFPE sections from a discovery cohort of 54 HCC patients with regions of interest selected in the tumor, interface and adjacent liver. We mapped stromal and parenchymal regions for each image. After channel normalization, cell segmentation, high-dimensional single-cell clustering and neighborhood analysis, we delineated the spatial immune architecture of the HCC TME. We validated the workflow in an independent cohort of 42 HCC patients that received ICI based therapies after tumor biopsy or resection and tested the new spatial classification regarding therapy outcome.

**Results:** Our approach identified several immune, stroma and tumor/hepatocyte cell clusters that reflect major cell types of the HCC and liver microenvironment. Unsupervised neighborhood detection based on spatial interaction of immune cells identified three immune neighborhoods with distinct cellular networks: (1) CD8 T cell dominant, (2) macrophage/granulocyte dominant and (3) B/CD4 T cell dominant neighborhoods. The variation of the neighborhood architecture revealed three major spatial subtypes that could be classified based on T cell infiltration and tumor compartments and that resemble conceptual features of immune-deplete, immune-excluded and immune-rich TMEs. We identified the differential contribution of distinct immune subsets between the spatial immune types. Analysis of the corresponding tumor interface regions showed clear differences in the immune network between the intratumor stroma and the capsule, pointing to different immunoregulatory mechanisms at the interface and the tumor stroma. Our findings could be validated in an independent cohort and progression-free survival under ICI based therapy differed significantly between the spatial immune types.

**Conclusion:** In sum, our in-depth spatial analysis successfully captured the immune heterogeneity of HCC patients. Spatial immune types may be reflective of different immune evasive strategies of the corresponding tumors and represent a potential novel biomarker for ICI based therapies.

## PO1-13
# Inhibition of the transmembrane transporter ABCB1 overcomes resistance to doxorubicin in patient-derived organoid models of hepatocellular carcinoma

Lauriane Blukacz[1], Sandro Nuciforo[1], Geoffrey Fucile[2], Fredrik Trulsson[1 2], Lukas Kübler[1], Urs Duthaler[1], Stefan Wieland[1], Markus Heim[1 3]

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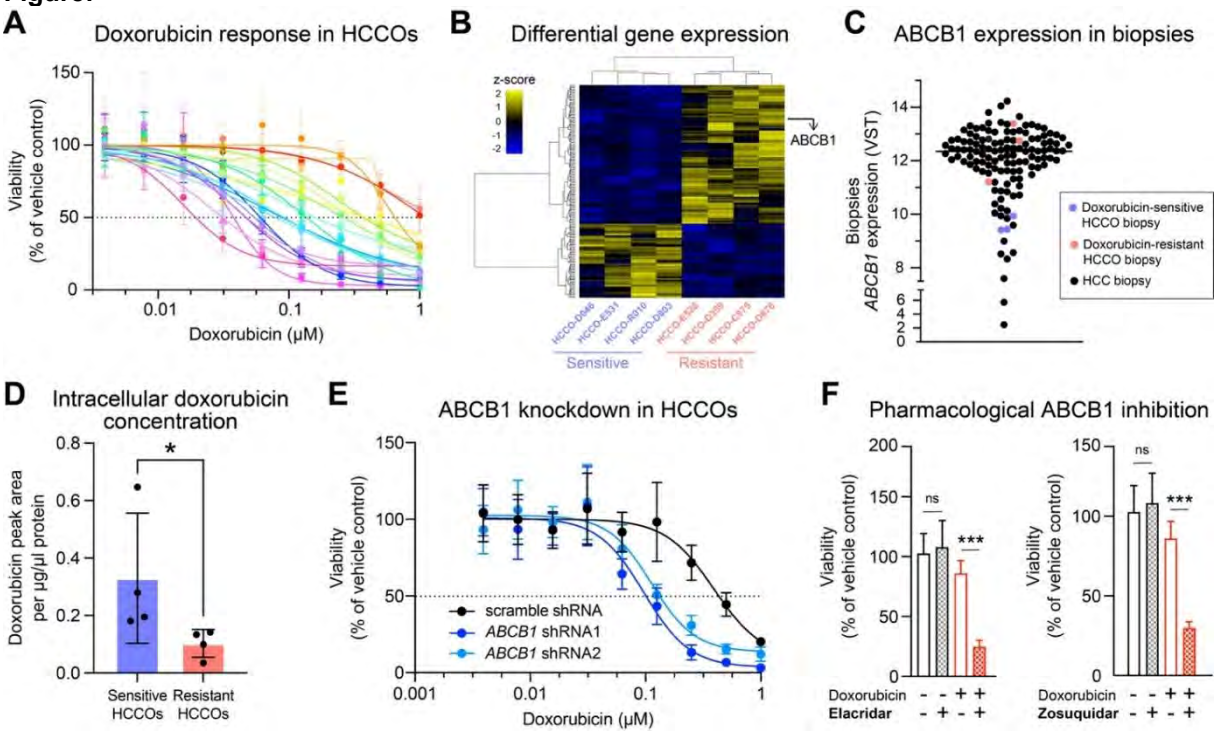
**Background and aims:** Response to transarterial chemoembolization (TACE) shows large interindividual variation. The molecular mechanisms underlying variable responses are poorly understood. Patient-derived HCC organoids (HCCOs) offer a novel platform to investigate variability of doxorubicin responses, the impact of hypoxia on tumor cell proliferation, and the molecular mechanisms underlying doxorubicin resistance.

**Method:** We evaluated the effects of hypoxia and doxorubicin on cell viability and cell cycle distribution in twenty patient-derived HCCO lines. Biocomputational analysis of transcriptome data were used to identify HCCO-intrinsic determinants of doxorubicin response. Candidate genes were validated by knock-down experiments and pharmacological inhibition.

**Results:** The IC50s of the doxorubicin response varied widely, from 29nM to >1µM. Doxorubicin and hypoxia did not exhibit synergistic effects but were additive in some HCCOs. Genes related to drug metabolism and export, most notably ABCB1, were differentially expressed between doxorubicin-resistant and sensitive HCCOs. In a series of 134 human HCC biopsies, we found that ABCB1 is commonly expressed at levels that impair response to doxorubicin. Intracellular concentration of doxorubicin was significantly lower in doxorubicin-resistant HCCOs with high expression of ABCB1. Knockdown of ABCB1 restored doxorubicin sensitivity in originally resistant HCCOs. Most importantly, inhibition of ABCB1 with elacridar and zosuquidar increased intracellular doxorubicin levels and thereby restored doxorubicin efficacy in resistant HCCOs.

**Conclusion:** ABCB1 overexpression is an important resistance mechanism of HCC cells contributing to non-response to doxorubicin. The pharmacological inhibition of the drug export pump ABCB1 is a promising strategy to increase response to TACE and should be further explored in clinical trials.

**Figure:**


A
Doxorubicin response in HCCOs
Viability (% of vehicle control)
Doxorubicin (μM)
B
Differential gene expression
z-score
ABCB1
Sensitive
Resistant
C
ABCB1 expression in biopsies
Biopsies ABCB1 expression (VST)
Doxorubicin-sensitive HCCO biopsy
Doxorubicin-resistant HCCO biopsy
HCC biopsy
D
Intracellular doxorubicin concentration
Doxorubicin peak area per µg/µl protein
Sensitive HCCOs
Resistant HCCOs
E
ABCB1 knockdown in HCCOs
scramble shRNA
ABCB1 shRNA1
ABCB1 shRNA2
F
Pharmacological ABCB1 inhibition
Doxorubicin
Elacridar
Zosuquidar
ns
***

## PO1-15

# N-acetylcysteine prevents experimental hepatocellular carcinoma through modulation of the Nrf2 signaling pathway and promotion of antifibrogenic and antiproliferative effects

Fernando Caloca Camarena[1] [2], Hugo Monroy Ramirez[1], Scarlet Arceo-Orozco[1], juan armendariz-borunda[1], Marina Galicia-Moreno[1]

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**Background and aims:** Hepatocellular carcinoma (HCC) is the most important primary liver cancer, and the third cause of death worldwide. Molecular mechanisms such as: oxidative stress, inflammation, fibrosis and cell proliferation play an important role in the hepatocarcinogenic process. The use of systemic chemotherapies in advanced HCC treatment remains limited. Currently, drug repositioning involves reusing an existing drug to treat a different disease than the one for which it was originally developed for. This approach reduces both cost and time required for pharmaceutical development. N-acetylcysteine (NAC) is a drug that is used clinically to treat drug-induced liver injury, but its ability to modulate molecular mechanisms that are activated during development of HCC is unknown. Our aim was to evaluate NAC hepatoprotective effects in an experimental HCC model.

**Method:** Male Fischer 344 rats were divided into 3 groups: 1. Control (CTL); 2. HCC: Diethylnitrosamine (DEN) + 2-acetylaminofluorene (2-AAF); 3. HCC/NAC: DEN+2-AAF and NAC treatment. Liver damage, oxidative stress, fibrosis and proliferation markers were evaluated by colorimetric assays, western blot, dot blot, immunofluorescence and immunohistochemistry respectively. Hematoxylin and eosin (HandE), Masson's trichrome (T.M), and Sirius red (S.R) stains were also performed. Data were expressed as mean values ± SD. Comparisons were carried out by analysis of variance followed by Tukey's test, as appropriate, using software GraphPad Prism 10. Differences were considered statistically significant when $p < 0.05$. This project was conducted in accordance with the guidelines of our institution under approval number CI-01723.

**Results:** NAC prevents serum markers of liver damage from increasing; histologically, HandE stains shows that HCC group has a great disruption of hepatic parenchyma and formation of dysplastic nodules, NAC slows down all these abnormalities. In addition, NAC suppress extracellular matrix accumulation, and attenuates alpha-SMA and TGF-beta expression. Regarding cellular proliferation markers, NAC declines glypican-3 and Ki-67 expression. Furthermore, NAC regulates Nrf2 signaling pathway, modulating downstream target proteins such as catalase, superoxide dismutase, heme oxygenase, and glutathione reductase. Finally, this drug prevents oxidative DNA damage through increasing 8-oxoguanine DNA glycosylase expression, and therefore drops 8-oxoguanine levels.

**Conclusion:** NAC efficacy in the prevention of HCC involves the modulation of oxidative, antifibrogenic, antiproliferative and antitumoral processes. Further analyses are needed to elucidate molecular mechanisms and safety of NAC to allow its repositioning as a co-adjuvant therapy in the treatment of HCC.

**Figure:**

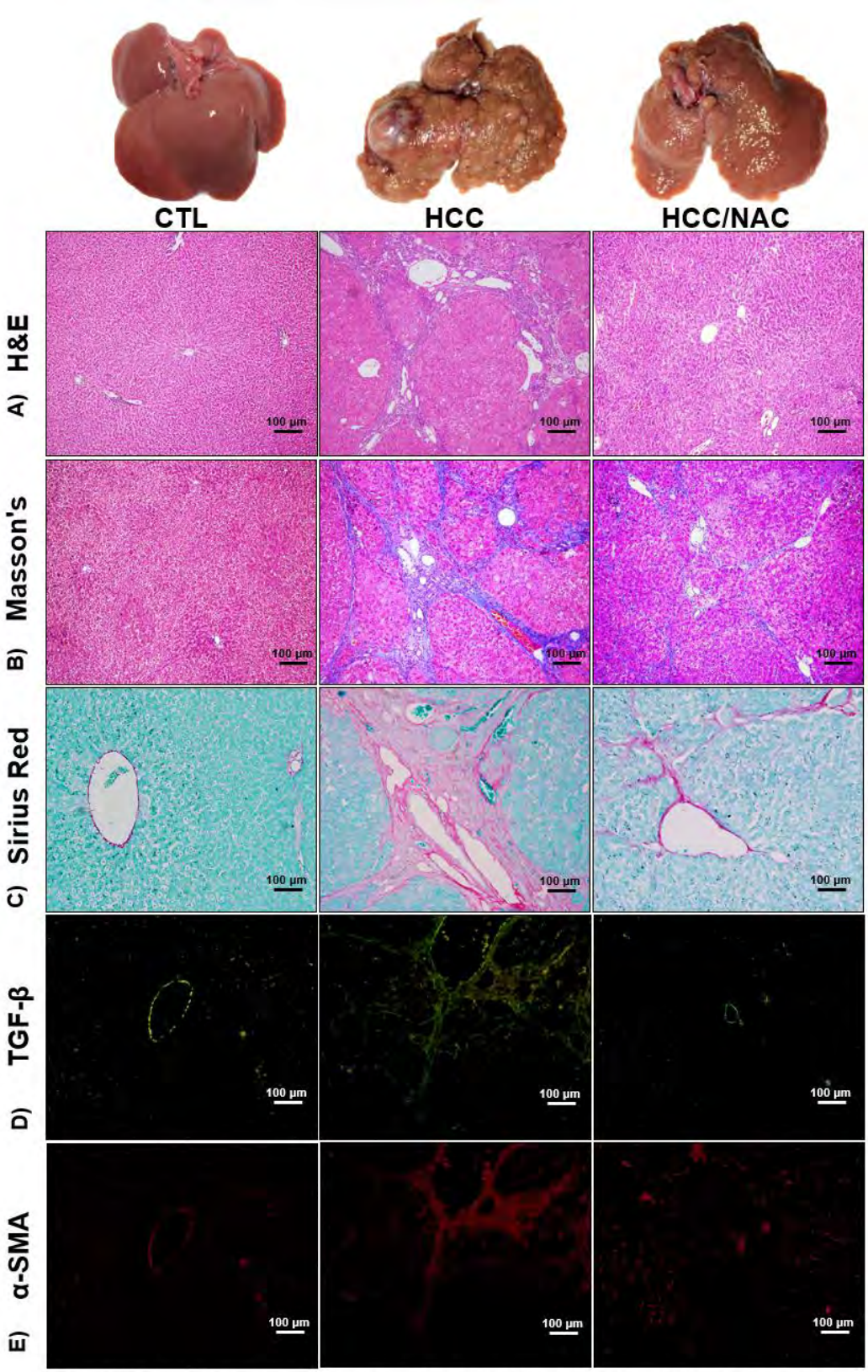


**Fig. 1.** NAC prevents damage to hepatic architecture. H&E stains shows that HCC group has a great disruption of hepatic parenchyma, and formation of dysplastic nodules; NAC slows down all these abnormalities (A). In addition, NAC possesses antifibrogenic capacity by suppressing of extracellular matrix accumulation (B, C), and attenuate profibrogenic markers increase such as TGF-β (D), and α-SMA (E). *H&E: Hematoxylin and eosin; MT: Masson's Trichrome; SR: Sirius Red.*

# PO1-18-YI
# Rare but not forgotten-comprehensive molecular and histological analysis of liver yolk sac tumor and corresponding patient-derived cell line

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**Background and aims:** Primary yolk sac tumor (YST) of the liver is uncommon disease with less than 15 cases reported worldwide and is often misdiagnosed as HCC. It is a type of germ cell tumor that arises from embryonic tissue left over from fetal development and may be initiated by the occurrence of genetic alterations or oncogenic mutations. Survival can vary widely depending on the stage of the tumor and the effectiveness of therapy. Therefore, in-depth molecular and histological characterization of YST and the development of representative models are essential to better understand and diagnose this disease and to develop patient-specific therapeutic options.

**Method:** Samples of adjacent liver and core tumor tissue were collected and processed from a 66-year-old female patient. Long-term culture of primary cell line (PCL) was established. Morphological and histological characteristics of the obtained tissues, xenograft tumors and cell line were analyzed by immunohistochemistry (IHC) and immunofluorescence (IF). Immune cell composition was inferred from RNA-seq data and validated by IHC. Transcriptomic profiling was performed by RNA sequencing followed by time-course, GSEA and IPA analyses. Key oncogenic alterations and potentially actionable mutations were identified by targeted NGS. Dose-response and synergistic effects between classical and targeted therapies were determined.

**Results:** Tumor tissue showed a solid trabecular growth pattern with the presence of necrosis. The newly derived cell line fully resembled the morphological features of the primary cancer in vitro and in vivo. IF and IHC showed expression of typical YST markers AFP, CK19, which were effectively maintained in xenografts, PCL and spheroids. Transcriptomic profiling revealed activation of embryonic markers, enrichment of T cells and reduction of macrophages and allowed identification of potentially effective therapeutics. In addition, the study revealed previously unknown genomic alterations and the presence of a novel driver mutation. Amplifications on chromosomes 22q12.2, 9q34.11, 8q24.3, previously associated with poorer survival in pancreatic neuroendocrine tumors, were detected, suggesting a potentially more aggressive disease. NGS approaches confirmed that key oncogenic mutation TP53 as well as potentially actionable mutation KDR were present in tumor and were highly conserved in PCL. Specific targeting of detected actionable mutation confirmed superior response and sustained sensitivity to specific inhibition compared to non-mutated control cells.

**Conclusion:** Here, for the first time, we were able to dissect the liver YST at an unprecedented molecular level and at the same time establish a primary cell line. We identified molecular alterations that could be used to effectively predict patient response to targeted therapy and serve as an effective model for this extremely rare disease.

# PO2-02
# Effect of atorvastatin together with rifaximin in the prevention of hepatocarcinogenesis generated by dioxin-type toxic

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**Background and aims:** Hepatocellular carcinoma (HCC) is the most common liver tumor. One of the main causes is exposure to dioxin-like compounds such as the endocrine disruptor hexachlorobenzene (HCB). A potential role of thyroid hormones (TH) in its development has been postulated, and statins prevent it. Rifaximin (RIF) has an anti-inflammatory capacity and reduces the expression of miRNAs involved in HCC. We show that HCB stimulates hepatic proliferation through TGF-β1 and TH, and that atorvastatin (AT) prevents it. To study the effects of HCB treatment on cell proliferation in Huh-7 cells, to analyze the ability of AT together with RIF to prevent such effects.

**Method:** Huh-7 line treated with HCB. Pretreatment with AT (30uM) and/or RIF (10uM) and/or exogenous TGF-B1 or T3. Specific objectives: A) Analyze: 1- proliferation (PCNA, cD1); 2-apoptosis (caspase-3 and Cytochrome-c); 3- cell regulation (TGF-β1, p27); 4- inflammation (TGF-β1 and Cox-2); 5- Metabolism of TH (DI) and avb3 receptors; 6- Cell migration. B) To study the prevention of AT with RIF, on the effect of HCB on the parameters listed in A. C) To analyze the role of TGF-β1 and T3 in the mechanism of preventive action of AT together with RIF, on HCB.

**Results:** HCB 5uM increased PCNA (39%, p <0.01), TGF-β1 (45%, p <0.01), cox-2 (25%, p <0.05), cytochrome-c (35%, p <0.01), caspase -3 (25%, p <0.05) and promotes cell migration (30%, p <0.01) compared to controls, western blot and wound test. The same effect was observed in the avb3 receptor (28%, p <0.05); on the contrary, DI decreased (26%p <0.01), RT-PCR. The pretreatment with AT and RIF prevented the increase of the mentioned parameters and decreased the cell migration generated by HCB (60%, p <0.01).

**Conclusion:** The dependence of HT on the preventive mechanism of AT has been demonstrated; however, the role of TH in the co-administration of AT and RIF remains unclear. Co-administration of AT and RIF shows non-additive antiproliferative, antimigratory and proapoptotic effects in preventing the development of HCC.

**Figure:**

***Co-administration of AT and RIF on cell proliferation***

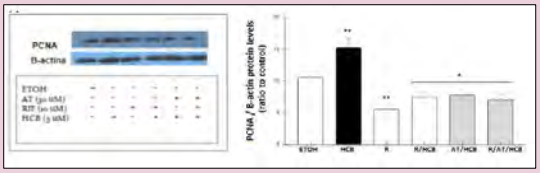


**Fig 1**. Effect of AT and RIFA on cell proliferation promoted by HCB Western blot analysis of total lysate of treated Huh-7 cells with 5 µM HCB, and pretreated with AT (30 µM) and/or RIFA (10 uM) for 3 hrs. Quantification of densitometric protein levels are shown in the right panel. Values are the mean ± SEM of three independent experiments. Significant differences (* p < 0.05 and **p < 0.01) compared to cells with HCB

***Co-administration of AT and RIF on cellular apoptosis***

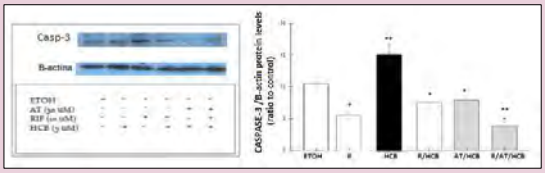


**Fig 2**. Effect of AT and RIFA on cell apoptosis promoted by HCB Western blot analysis of total lysate of treated Huh-7 cells with 5 µM HCB, and pretreated with AT (30 µM) and/or RIFA (10 uM) for 3 hrs. Quantification of densitometric protein levels are shown in the right panel. Values are the mean ± SEM of three independent experiments. Significant differences (* p < 0.05 and **p < 0.01) compared to cells with HCB

# PO2-07
# Canonical and non-canonical activities of cGAS have opposite effects in liver tumorigenesis

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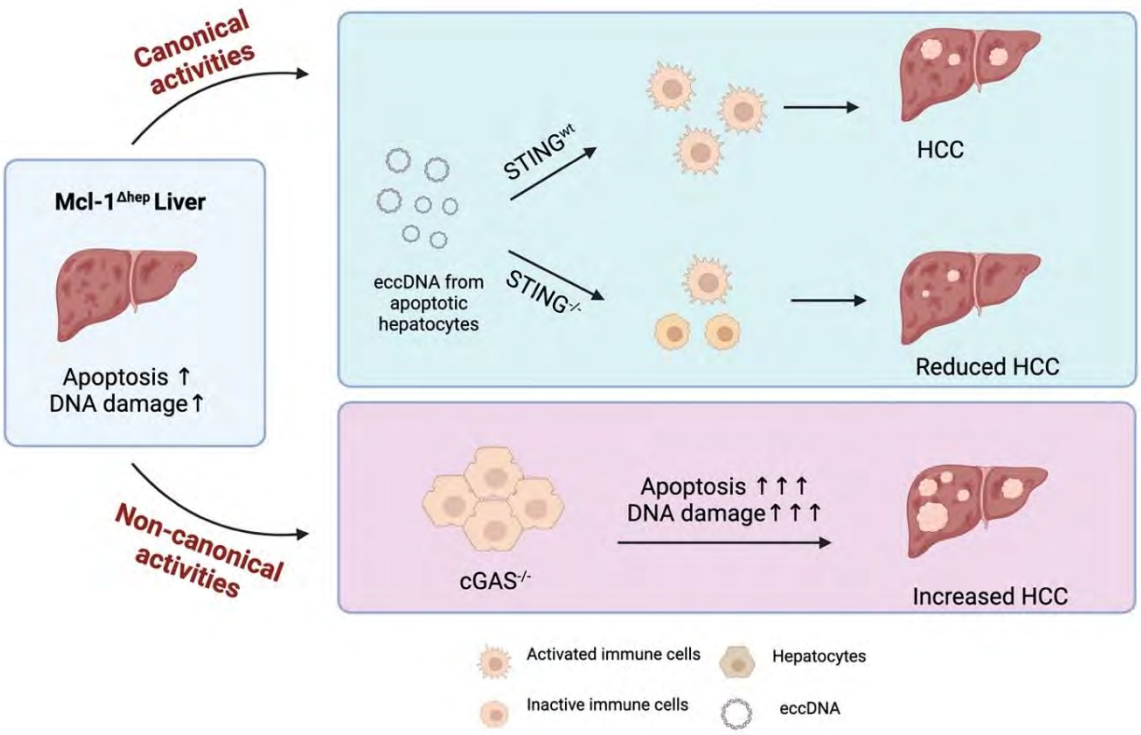
**Background and aims:** Hepatocellular carcinoma (HCC) mostly develop on the background of a chronic liver disease (CLD). The hallmarks of CLD include increased hepatocyte apoptosis, compensatory proliferation and DNA damage. Mice with an ablation of myeloid-cell leukemia 1 (MCL-1) specifically in liver parenchyma (Mcl-1$^{\Delta hep}$ mice) recapitulate key features of CLD and subsequently develop HCC later in life. However, it is incompletely understood how MCL-1 deficiency drives liver tumorigenesis, in particular if this is caused by a cell autonomous effect.

**Method:** We analysed replication stress and the activation the cGAS-STING pathway in Mcl-1$^{\Delta hep}$ mice. We isolated extrachromosomal circular DNA (eccDNA) from Mcl-1$^{\Delta hep}$ liver, as well as in apoptotic AML12 cells, and studied their properties in inducing immune response. We also generated Mcl-1$^{\Delta hep}$/STING-/- and Mcl-1$^{\Delta hep}$/cGAS-/- double knockout mice to study the effects of cGAS and STING in liver homeostasis and tumorigenesis.

**Results:** Mcl-1$^{\Delta hep}$ mice showed increased replication stress, micronucleated hepatocytes and cytosolic DNA sensing at 2 months of age. Immunohistochemical analyses indicated that both hepatocytes and non-parenchymal cells (NPCs) expressed cGAS, whereas STING expression was restricted to NPCs. We found that a higher level of apoptosis in Mcl-1$^{\Delta hep}$ liver increased the generation and accumulation of eccDNAs. eccDNAs acted as a mediator to promote a crosstalk between hepatocytes and immune cells in driving the immune response which was dependent on the canonical cGAS-STING pathway. In fact, deletion of STING in Mcl-1$^{\Delta hep}$ mice reduced immune cell chemotaxis, as well as tumor incidence. In contrast, deletion of cGAS resulted even higher apoptosis, DNA damage and proliferation compared to Mcl-1$^{\Delta hep}$ mice, consequently leading to a higher tumor incidence. We observed that hepatic nuclear translocation of cGAS was significantly increased in Mcl-1$^{\Delta hep}$ mice and positively correlated to a higher proliferation rate. Since both cGAS and STING are essential in activating the canonical pathway, the protective function of cGAS in hepatocytes is most likely associated with its non-canonical functions in the nucleus.

**Conclusion:** Our findings indicate that cGAS and STING play different functions in liver carcinogenesis. eccDNAs generated from increased apoptosis in Mcl-1$^{\Delta hep}$ liver is, at least partly, contributing to the crosstalk between hepatocytes and immune cells. This drives liver tumorigenesis, suggesting a non-cell autonomous mechanism. Our results also have implications for considering using cGAS inhibitors, as an alternative to STING inhibitors, to regulate immune response in HCC.

**Figure:**


Canonical activities
Mcl-1Δhep Liver
Apoptosis ↑
DNA damage ↑
Non-canonical activities
eccDNA from apoptotic hepatocytes
STINGwt
STING-/-
HCC
Reduced HCC
cGAS-/-
Apoptosis ↑ ↑ ↑
DNA damage ↑ ↑ ↑
Increased HCC
Activated immune cells
Inactive immune cells
Hepatocytes
eccDNA

# PO2-09-YI
# Investigating the immunogenetic heterogeneity in hepatocellular carcinoma

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**Examining the immunogenetic heterogeneity in hepatocellular carcinoma**

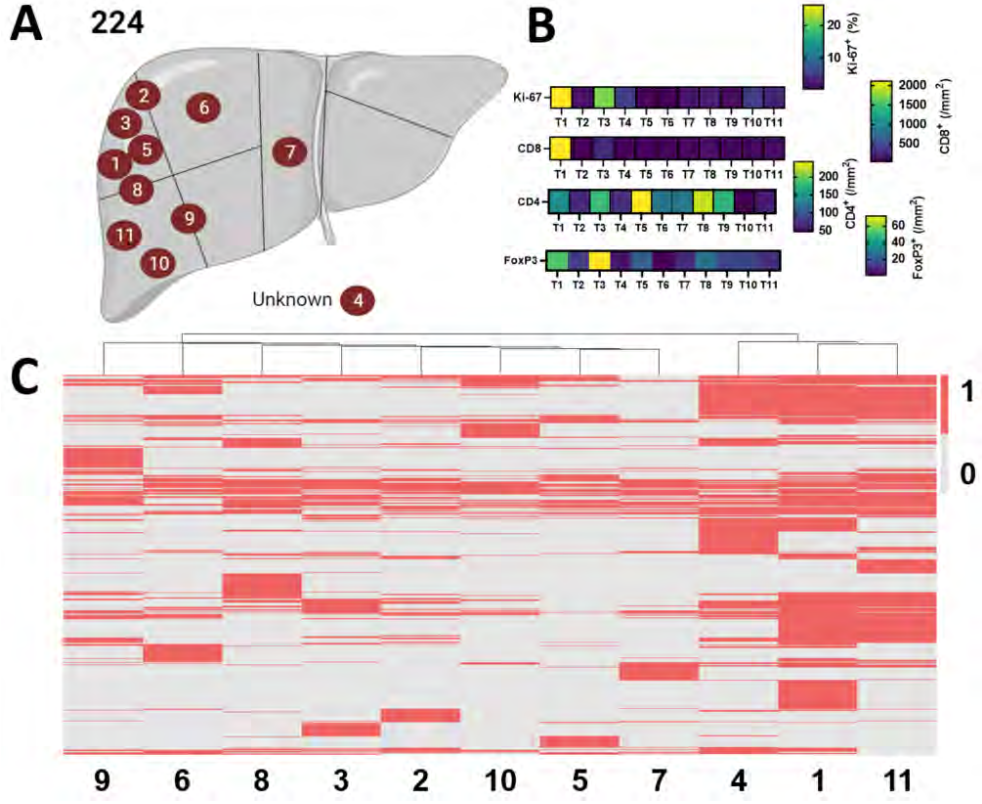
**Background and aims:** Since 2020, NICE guidelines have recommended concurrent immunotherapy of atezolizumab and bevacizumab for advanced, non-resectable, hepatocellular carcinoma (HCC) in the UK. This offers favourable outcomes compared to previous treatment options, however it is not currently understood why treatment benefit is restricted to less than one third of patients. My PhD research aims to investigate the genetic and immunological differences that exist within the HCC patient population, to better understand this discrepancy in treatment response.

**Method:** FFPE blocks representing 73 tumours and paired background liver samples from 27 patients were obtained from the NHS, preserved after hepatectomy following HCC diagnosis. Whole exome sequencing was performed on paired samples to identify tumour-specific variants and enable the calculation of tumour mutation burden (TMB). In parallel, sections from these same blocks were stained for HandE, Ki-67 and markers for T cell infiltration, using quantitative immunohistochemical techniques.

**Results:** These results confirm previously reported findings that HCC tumours have an intermediate TMB, compared to other tumour types, however substantial variation does exist between tumours. This heterogeneity was also observed in the immune cell infiltration, with only FoxP3+ regulatory T cells being significantly enriched in tumour samples. In the 10 cases of multifocal disease, the level of variant similarity indicates a mixture of patients with intrahepatic metastases and multicentric occurrence. Despite tumours being of metastatic relation, a discrepancy in immune cell infiltration is observed.

**Conclusion:** There is a high level of heterogeneity within the HCC patient population that cannot be accounted for using existing patient or tumour classification groups. This variation also exists in multifocal disease, with tumours that are genetically related exhibiting opposing T cell infiltration patterns. Overall, this research confirms that HCC patients represent a diverse mutagenic landscape and do not frequently have high immune cell infiltration. This supports a hypothesis where, in a significant proportion of patients, a low number of neoepitopes could be limiting the efficacy of immunotherapies that rely on an existing tumour response. This supports the need for personalised treatments and in particular those that are focused on priming the immune response.

**Figure:**




**Immunogenetic profiling of multifocal HCC (ID 224).**

A) Location of multifocal tumours, according to liver segment.
B) Summary of tumour proliferation and immune cell infiltration, quantified using QuPath.
C) A clustered heatmap showing variants that are shared between tumours.

## PO2-18-YI

# The potential modulatory role of Aurora Kinase A on yes-associated protein and Glycogen synthase kinase-3 beta in advanced chronic liver disease

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**Background and aims:** Aurora Kinase A (AURKA) is a well-recognized oncogene in many cancer types, including hepatocellular carcinoma (HCC), and is primarily involved in the cell's mitotic machinery. Advanced Chronic liver diseases (ACLD), particularly cirrhosis, is characterized by a high rate of proliferation to compensate for hepatocyte loss. Thus, the canonical role of AURKA in mitosis may provide clues on nuanced role of AURKA in this part of the diseased liver, which has not been described yet in literature. The functional hepatocyte loss is accompanied by hepatic stellate cell (HSC) activation responsible for the increased deposition of extracellular matrices (ECMs) that eventually lead to fibrotic scars. These repeated processes are regulated by several pathways including Hippo and Wnt cascades. In ACLD context, this study aims to probe the regulatory role of AURKA on two of its reported phospho-targets, YAP (Ser397) and GSK-3β (Ser9), key components of Hippo and Wnt signaling, respectively. The findings may provide clues on AURKA's activity and lead to additional therapeutic targets in the disease.

**Method:** Quantitative Real-Time PCR (RT-qPCR) and Western Blot were used to evaluate the mRNA and protein expression of AURKA, YAP, and GSK-3β in HCC nodules and paired non-tumoral tissue samples with ACLD collected from HCC-resected livers. Immunohistochemistry (IHC) staining was used to validate the expression of AURKA protein in the tissues.

**Results:** Our data revealed upregulated AURKA expression ($p < 0.001$) within the paired non-tumoral liver tissues ($n = 54$) in contrast to the nodular tissues ($n = 54$) excised from HCC-resected livers, suggestive of a distinctive role for AURKA in non-neoplastic chronic liver disease. Consequently, we probed for mRNA and protein levels of YAP and GSK-3β using RT-qPCR and western blot, respectively. Our results revealed an elevated YAP protein expression ($p < 0.01$) in the non-tumoral liver tissues relative to the nodules from these HCC tumors, while GSK-3β protein ($p < 0.0001$) displayed an inverse trend. Nodular and non-tumoral protein expressions of AURKA positively and negatively correlate with YAP ($p < 0.0001$) and GSK-3β ($p < 0.0001$), respectively. Expectedly, *YAP* mRNA showed no significant difference in the two tissue groups, supporting our hypothesis that AURKA predominantly modulates YAP protein, while *GSK-3β* mRNA ($p < 0.01$) showed significantly lower levels in the paired-non cancerous tissues.

**Conclusion:** AURKA is significantly upregulated in ACLD compared to the paired HCC tissue. Its phospho-targets, YAP and GSK-3β, correlate with AURKA expression in both tissue groups. YAP and GSK-3β protein expressions, so far, agree with the reported modulatory mechanism of AURKA on these two targets. The regulatory role of AUKRA over these targets may thus provide further information regarding the relevant role of AURKA in ACLD development and progression.

# PO3-01-YI
# Role of Nrf2 signaling in the crosstalk between Trx1 and GSNOR-redox regulators in liver cancer cells

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**Background and aims:** Hepatocellular carcinoma (HCC) constitutes the sixth most common type of cancer and the fourth leading cause of cancer-related death globally. Sorafenib is a multikinase inhibitor useful to treat patients in advanced-stage of hepatocellular carcinoma (HCC) who cannot benefit from the first-line treatment consisting of Atezolizumab and Bevacizumab. We have previously shown that the proapoptotic and antiproliferative properties of Sorafenib activities are associated with altered cellular redox status, and downregulation of Nrf2-related thioredoxin-1 (Trx1) expression and activity in liver cancer cells. S-nitrosoglutathione reductase (GSNOR) is involved in the regulation of cellular S-nitrosation process. The aim of the study was the identification of the crosstalk among redox and S-nitrosation regulations in liver cancer cells during Sorafenib treatment in liver cancer cells.

**Method:** We determined the impact of Nrf2 and/or regulation of GSNOR expression by siRNA in cell death and proliferation, cell migration and invasiveness by Sorafenib in 2D cultured liver cancer cells. Sorafenib (10 μM) was administrated to HepG2. shSCR- and shGSNOR-transfected HepG2 cells were used to elucidate the role of GSNOR. Nrf2 was induced using Sulforaphane (10 μM) in HepG2 cells. We assessed GSNOR, Nrf2, Trx1, NQO1, vimentin, slug, and E-cadherin/N-cadherin ratio by Western-blot analysis. Apoptosis, cell proliferation, cell migration and invasiveness were determined using different experimental procedures.

**Results:** The reduction of cell proliferation, migration and invasiveness by Sorafenib was related to downregulation of all markers of epithelial mesenchymal transition (EMT), Nrf2 and GSNOR protein expression. Sulforaphane increased Trx1 and NQO1 protein expression in HepG2. Interestingly, although the activation of Nrf2 by Sulforaphane transiently increased mRNA GSNOR expression, its protein content was reduced by Sulforaphane in control cells, Sulforaphane increased cell migration and invasiveness in control cells. Sorafenib was able to prevent the increase of cell migration and invasiveness until 36 h of treatment. Interestingly, the sustained Sulforaphane administration over 36 h overcame Sorafenib-antitumoral properties.

**Conclusion:** 1) Sorafenib reduced Nrf2-dependent signalling and GSNOR expression in liver cancer cells. 2) The regulation of GSNOR expression by Sulforaphane suggests a relevant crosstalk between redox regulation and nitrosative stress. 3) Sustained Sulforaphane treatment overcame antitumoral properties of Sorafenib.

# PO3-03-YI
# MAP17 stimulates mitochondrial activity by enhancing one-carbon metabolism, leading to the promotion of EMT in hepatocellular carcinomas (HCCs)

Claudia Gil-Pitarch[1], Iker Uriarte[2], Esther Bertran[3], Natalia Hermán-Sánchez[4], José Manuel García-Heredia[5], Rubén Rodríguez Agudo[1], Naroa Goikoetxea[1], Maria Mercado-Gómez[1], Irene González-Recio[1], Teresa Cardoso Delgado[1], Maria Vivanco[6], Luis Alfonso Martinez-Cruz[1], Cesar Augusto Martín[7], Rafael Artuch[8], Mario Fernández[9], Manuel Gahete Ortiz[4], Isabel Fabregat[3], Matías A Avila[2], Amancio Carnero[10], María Luz Martínez-Chantar[1 11]

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**Background and aims:** Epithelial-mesenchymal transition (EMT) a crucial process in embryonic development, facilitates cell migration and provides resistance to apoptosis during tumour invasion and metastasis. In hepatocellular carcinoma (HCC) an amoeboid behaviour intensifies the aggressiveness and metastatic potential of epithelial tumours. MAP17, a membrane protein expressed in embryonic stages, is typically absent in most adult organs. Its presence in tissues correlates with an inflammatory environment, hypoxia, and elevated reactive oxygen species (ROS). MAP17 has been identified in various cancer types, including HCC. Modulating EMT and amoeboid behaviour through MAP17 represents a promising strategy to hinder metastasis.

**Method:** Two separate cohorts of HCC patients were employed to analyse MAP17 levels. In vitro, MAP17 expression was examined in mesenchymal and epithelial hepatoma cells. Its levels were altered to investigate cell proliferation, mitochondrial dynamics, metabolic changes, and proteome stability. The influence of MAP17 on metastatic potential was assessed in vivo using orthotopic HCC mouse models.

**Results:** In 751 HCC patients, in silico studies and mRNA expression analysis established a positive correlation between MAP17 and mesenchymal markers and RAC/RHO family genes, indicators of amoeboid movement. Furthermore, MAP17 overexpression in vitro caused a reprogramming of energy metabolism in hepatoma cells with epithelial characteristics which increased mitochondrial dynamics and lactic acidosis with a concomitant ROS production, creating a tumor microenvironment conducive to cancer cell proliferation. Additionally, a reconfiguration of the one-carbon metabolic pathway was identified, resulting in accelerated cell metabolism. This included faster methionine degradation fueling the folate cycle, a source of purines and pyrimidines, supporting a highly proliferative state.
The presence of MAP17 modulates hypomethylation of HLF and HNF4A promoters, allowing its expression. These genes could be the key in the induction of epithelial-mesenchymal-ameboid transition, as they act as mesenchymal gene transcription factors and are involved in the progression of HCC. In line with these findings, overexpressing MAP17 in PLC/PRF/5 cells led to the formation of multiple tumor foci when implanted orthotopically in the mouse liver. Silencing MAP17 in hepatoma cells with mesenchymal traits yielded opposite results, causing regression in the tumor phenotype and slowing down cell metabolism and proliferation.

**Conclusion:** Modulating MAP17 in epithelial and mesenchymal HCC cells instigates changes in the transitional genes that delineate each phenotype. Our investigations have identified MAP17's metastatic potential in liver cancer, showcasing its ability to initiate the mesenchymal phenotype and promote amoeboid behaviour in HCC.

## PO3-05-YI

# 1-Piperidine propionic acid is effective in reducing HCC development and fatty acid accumulation in experimental liver carcinogenesis

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**Background and aims:** hepatocellular carcinoma (HCC) and metabolic dysfunction-associated steatotic liver disease (MASLD) are two major problems in modern hepatology since their incidence is increasing and specific treatments are lacking. Protease-activated receptor 2 (PAR2) is a member of G protein-coupled receptors and has been associated with lipid metabolism dysregulation and cancer progression, becoming an interesting therapeutic target. Recent results indicate that PAR2 is activated by SerpinB3, a serin-protease inhibitor involved in fibrosis and oncogenesis and is inhibited by the small molecule 1-Piperidine propionic Acid (1-PPA). This study aims to investigate the effect of 1-PPA in steatosis progression and HCC development.

**Method:** in a mouse model of liver carcinogenesis, wild-type (BC/WT), knocked-out (BC/KO) and transgenic (C57/TG) mice for SerpinB3 were injected with diethylnitrosamine (DEN) and fed with CDAA diet in the presence or absence of 1-PPA for 26 weeks. Human preadipocytes were cultured in vitro with different concentrations of SerpinB3 (SB3) and 1-PPA. Primary hepatocytes extracted from C57 wild-type mice were treated with different steatogenic conditions (oleic acid, methionine-choline deficient (MCD) and SB3), different concentrations of 1-PPA and/or an inhibitor of VLDL formation/export: lomitapide.

**Results:** the presence of SerpinB3 in mice was associated with larger liver tumours, showing high steatosis content and this effect was reverted by 1-PPA. Mice treated with 1-PPA showed liver reduction of ER stress-related genes and in primary hepatocytes, this compound determined a reduction of ROS accumulation. Proteomic analysis revealed that 1-PPA determined a reduction in lipid metabolism and cancer-development-associated pathways. Human preadipocytes treated with SerpinB3 showed increased production of pro-adipogenic C/EBP-beta which was inhibited by administration of 1-PPA. Primary hepatocytes treated with steatogenic conditions and 1-PPA showed a reduction in lipid body accumulation, compared to controls. Simultaneous treatment with lomitapide erased the effect of 1-PPA, suggesting the involvement of VLDL formation/export in the mechanism of 1-PPA-associated lipid reduction. In agreement, 1-PPA treatment in mice was associated with higher levels of triglycerides in serum.

**Conclusion:** PAR2 inhibition by 1-PPA leads to reduced liver cancer development, associated with lower lipid accumulation, favoured, at least in part by an increased VLDL secretion.

# PO3-06
# Transcriptomic landscape of epigenetic effectors in human cholangiocarcinoma

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**Background and aims:** Epigenetic alterations are increasingly recognized as pro-tumorigenic in cholangiocarcinoma (CCA). The epigenetic machinery includes genes (EpiG) functionally classified as writers, erasers and readers of epigenetic marks. Importantly, epigenetic enzymes' activity depends on the levels of key metabolites linked to one carbon metabolism (OCM), tricarboxylic acid cycle (TCA) and acetyl-CoA synthesis (ACS). We comprehensively analyzed the expression of EpiG and metabolic genes (MG) in human CCA tissues alongside previously reported prognostic transcriptomic signatures. We also tested the dependency of CCA cells lines on the expression of a broad complement of EpiG.

**Method:** We analyzed the expression of 257 EpiG and 89 MG. RNAseq data from intrahepatic CCA (iCCA, n = 285), extrahepatic CCA (eCCA, n = 46) and normal intrahepatic bile duct tissues (NBD, n = 7) were integrated. Microarray data from iCCA (n = 217), eCCA (n = 208) and NBD (n = 50) tissues were also analyzed. The impact on CCA cells viability of 248 EpiG was inferred from CRISPR-Cas9 data (DepMap, n = 28 cell lines).

**Results:** A significant number of EpiG from all 3 categories were upregulated in iCCA and eCCA tissues, including writers like *DNMT1*, *EHMT2*, *EZH2*, *CARM1*, *KAT2A*, *MECOM*, *SMYD2*; readers *BAZ2B*, *BRWD3*, *DMAP1*, *CBX5*, *UHRF1*, *PHF20L1, ING2*; and erasers *HDAC1*, *HDAC3*, *HDAC8*, *HIF1AN*. In iCCA several EpiG were associated with the proliferative subclass (*e.g. MBD6*, *SUV39H1*, *MSH6*, *HDAC1*), the inflammation subclass (*e.g. PRMT7*, *SETDB1*) or both (*e.g. UHRF1*, *BAZ2B*, *CBX5*, *EZH2, HDAC3*). Expression of 17 EpiG, like *HDAC1*, *HDAC3*, *ING2* and *MSH6*, negatively correlated with iCCA patients' survival, and most of them were also overexpressed. DepMap analysis identified over 40 EpiG which inhibition reduced the viability of ~90% of CCA cells. Several MG involved in the OCM, TCA and ACS pathways were upregulated (*e.g. ACHY*, *SHMT2*, *DLAT*, *SDHC*, *ACSS2, IDH1, IDH3G, GART, MTHFD2*) or downregulated (*e.g. MAT1A*, *BHMT*, *GNMT*, *PCK2*) in CCA tissues. The expression of some of these MG (*e.g. IDH3G*, *MTHFD2*) negatively correlated with iCCA patients' survival.

**Conclusion:** There are marked changes in EpiG expression in human CCA. Specific EpiG were particularly overexpressed in the more aggressive proliferative molecular subclass, in association with poor patients' prognosis. We found a marked rewiring of the expression of epigenetic cofactors-related MG in CCA. Functional assays validated new EpIG targets in CCA.

# PO3-07-YI
# Role of nitric oxide in cell resistance to Sorafenib in HepG2 cell line

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**Background and aims:** Sorafenib is a multikinase inhibitor useful to treat patients in advanced-stage of hepatocellular carcinoma (HCC) who cannot benefit from the first-line treatment Atezolizumab+Bevacizumab. Sorafenib induces endoplasmic reticulum (ER) stress, mitochondrial dysfunction, apoptosis and reduced cell proliferation in liver cancer cells. Nitric oxide exerts pro- or anti-tumoral properties in a dose-, time- and compartment-dependent manner. The aim of the study was to determine the role of nitric oxide (NO) in parenteral and Sorafenib-resistant HepG2 cells (R-HepG2).

**Method:** NO production was inhibited by administration of N (ω)-nitro-L-arginine methyl ester (L-NAME) (3 mM) in Sorafenib (10 μM)-treated cells. Autophagy, apoptosis, cell proliferation and respiration, and NO were assessed. S-nitrosylated (SNO) proteins were purified by biotin-switch assay and identified by mass spectrometry.

**Results:** R-HepG2 showed a shift from the PERK and ATF6 to IRE-related ER stress, increased cell proliferation, cell respiration, autophagy, and reduced caspase-3 activity and NO/SNO content compared to HepG2 cells. L-NAME increased the endoplasmic reticulum stress, autophagy and cell proliferation in HepG2 cells. The identification of the top differentially SNO proteins by mass spectrometry showed that Sorafenib significantly increased the abundance of 66 SNO proteins, while resistance to Sorafenib reduced the abundance of 77 SNO proteins ($p \leq 0.05$). The ulterior selection of the altered proteins showed that Sorafenib impacted the *Staphylococcus aureus* infection (3/31 genes), estrogen signaling pathway (3/31), cell cycle (2/31), mitophagy-animal (2/31), proteoglycans in cancer (3/31), spliceosome (4/31), hippo signaling (2/31) and oocyte meiosis (2/31) pathways in HepG2 cells ($p \leq 0.10$). Furthermore, the resistance to Sorafenib altered hepatitis C diseases (6/44), Herpes simplex virus 1 infection (5/44), cell cycle (4/44), hippo signaling (4/44), oocyte meiosis (4/4), hepatitis B infection (4/4), alcoholic liver disease (4/44), viral carcinogenesis (5/44), PI3K-Akt signaling pathway (4/44), fatty acid metabolism (3/44), tuberculosis (3/44), biosynthesis of amino acids (4/44), human cytomegalovirus infection (3/44), protein processing in ER (5/44) pathways in HepG2 cells ($p \leq 0.10$).

**Conclusion:** 1) The reduction of NO/SNO generation participated in the mechanisms of resistance to Sorafenib in R-HepG2 cells. 2) The induction of resistance to Sorafenib was associated with an alteration of the pattern of ER stress pathways. 3) Sorafenib and its resistance altered the pattern of SNO protein abundance which impacted different pathways involved in cell signaling and disease development.

## PO3-08
# Cholangiocarcinoma patient-derived organoids: an in vitro platform for antibody-drug conjugates sensitivity testing

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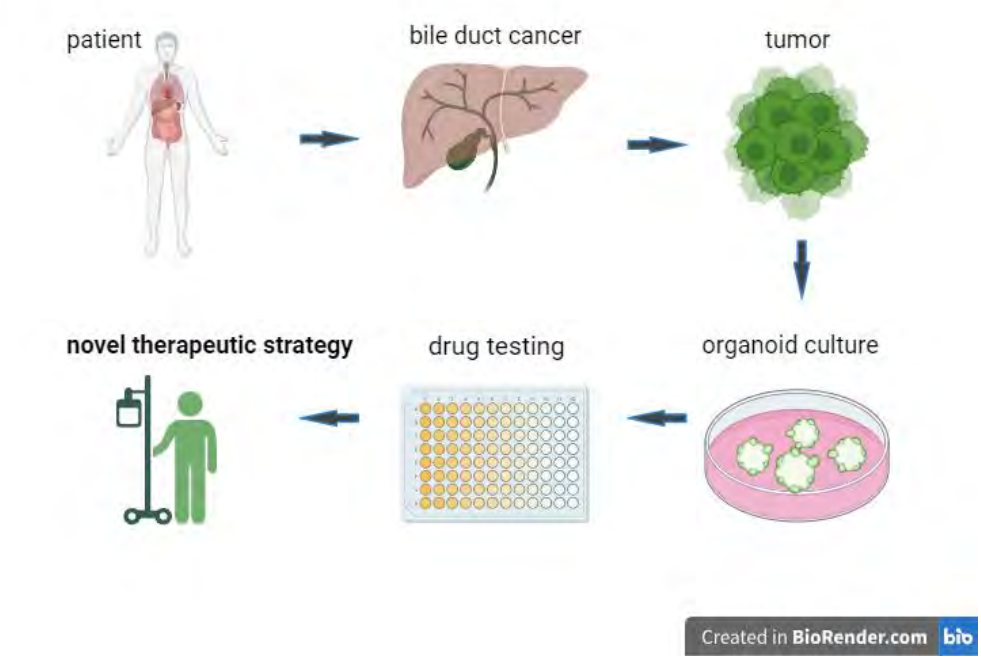
**Background and aims:** Cholangiocarcinoma is a rare cancer type with very limited therapeutic options. Tumor organoids, also known as patient-derived organoids (PDOs), are cultured from a tumor biopsy or resection specimen, and closely recapitulate key aspects of the tumor from which they were derived. Accordingly, PDOs represent a physiologically-relevant preclinical model for efficient translation of basic research to clinical regimens. This study aimed to establish PDO cultures from cholangiocarcinoma tumors and use them as a model for testing recently-approved antibody-drug conjugates.

**Method:** We established PDO cultures from four cholangiocarcinoma patients and tested their response *in vitro* to the antibody-drug conjugates sacituzumab govitecan (SG) and enfortumab vedotin (EV). We further investigated the expression profile of the antibody targets Trop2 and Nectin 4 via immunohistochemistry (IHC) in non-neoplastic liver ($n$ = 9) and gall bladder samples ($n$ = 20) and in a cholangiocarcinoma cohort ($n$ = 52). Thereafter, we analyzed the expression level in the cholangiocarcinoma cohort with respect to tumor localization, clinicopathological parameters, and patient survival.

**Results:** PDO cultures were responsive to the anti-tumor activity of SG, but not to EV. Trop2 IHC analysis showed a heterogenous, moderate, and strong expression in non-neoplastic inflammatory gall bladder mucosa, intrahepatic bile ducts, and hepatic ductular reactive proliferates, respectively. Moderate to strong Trop2 expression was observed in 34/52 (65%) of the cholangiocarcinoma cases. High Trop-2 expression was more frequently observed in intrahepatic cholangiocarcinoma than in extrahepatic cholangiocarcinoma. Trop2 expression did not correlate with overall or disease-specific survival and was significantly higher in lower-staged tumors (pT1/2) and in tumors without lymph node metastases. Nectin 4 expression was analyzed in 48 cases. Nectin 4 was positive in small cytoplasmic spots, mostly in a part of tumor cells. 20 cases were negative, 20 cases were low positive, and only 8 cases were moderately positive for Nectin 4. No association between Nectin 4 expression and clinicopathological data was noticed.

**Conclusion:** This study demonstrates the feasibility of using PDO cultures as *in vitro* models for antibody-drug conjugates testing. Moreover, it provides preclinical evidence for the potential for use of SG as a new therapeutic strategy in treating cholangiocarcinoma patients, especially those harboring Trop2 positive tumors.

**Figure:**


patient
bile duct cancer
tumor
novel therapeutic strategy
drug testing
organoid culture
Created in BioRender.com bio

# PO3-12-YI
# Circulating vesicle microRNAs for metabolic dysfunction-associated steatotic liver disease (MASLD) staging and progression towards liver cancer

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**Background and aims:** Non-invasive biomarker-driven algorithms are urgently needed for classifying Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) and monitoring its progression to Steatohepatitis (MASH) and Hepatocellular Carcinoma (HCC). In this study, we investigated microRNAs (miRs) present in serum extracellular vesicles (EVs) that may serve as diagnostic and prognostic biomarkers.

**Method:** EV-associated miRNAs from obese adults without Steatotic Liver Disease (SLD), lean or obese individuals with simple steatosis, MASH with and without fibrosis (determined by liver biopsy), as well as from patients with MASLD-associated HCC were isolated following the exoRNeasy Midi kit (Qiagen). The small RNA transcriptome on EVs was sequenced following the QIAseq miRNA Library kit (Qiagen) workflow.

**Results:** A total of 71 individuals were clinically categorized into eight study groups (n = 9 individual per group): Obese without SLD, with simple steatosis, MASH-F0/F1 or MASH-F2-F3; lean with simple steatosis, MASH-F0/F1 or MASH-F2-F3; and MASLD-HCC. In total, 1, 461 EV-miRs were identified. Notably, the EV levels of 8 miRs were found to be dysregulated in MASH compared to simple steatosis, irrespective of the degree of liver fibrosis and BMI, with individual areas under the receiver operating curve (AUC) values over 0.8. Additionally, the EV levels of 6 miRs were associated with fibrosis (F0-F1 vs. F2-F3) regardless of BMI, with AUC values over 0.83. Finally, the abundance of a 130 miRs were found to be altered in serum EVs from patients with MASLD-HCC compared to MASLD, regardless of age, biological sex, and BMI, with some of them exhibiting maximal diagnostic AUC values (e.g., miR-629-5p, miR-488-5p, miR-4732- 5p).

**Conclusion:** This study underscores the potential of EV-miRs as diagnostic tools for staging MASLD and its association with HCC. Ongoing work on logistic models that combine miRs will be valuable for future international validation efforts.

## PO3-13-YI

# Combined extracellular matrix remodelling by cyclophilin inhibitor rencofilstat and immune checkpoint blockade as a potential therapy for intrahepatic cholangiocarcinoma

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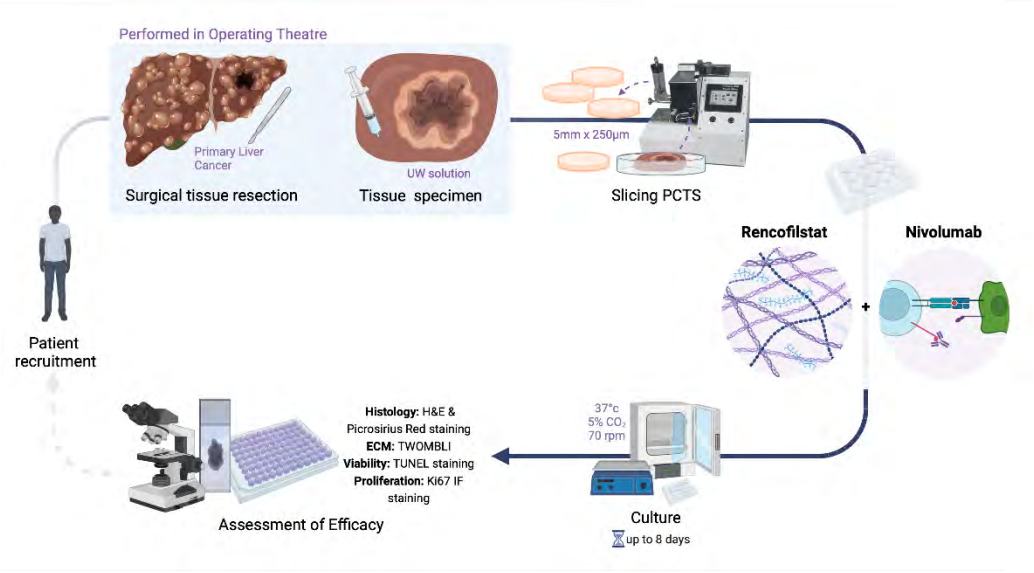
**Background and aims:** Advances in cholangiocarcinoma (CCA) therapy have not been mirrored by a substantial improvement in overall survival in the past decade, representing an unmet need in cancer research. Intrahepatic cholangiocarcinoma (iCCA) is characterised by a dense fibro-collagenous-enriched extracellular matrix (ECM), where the interplay between cancer cells and other cell types contributes significantly to tumour progression, therapeutic resistance, and immune evasion. Targeting the tumour microenvironment (TME) with multiagent approaches has produced promising results in recent studies in liver cancers. The inhibition of cyclophilins, isomerases overexpressed in fibrosis and associated with poor prognosis in various cancers, has shown beneficial effects in preclinical models of hepatocellular carcinoma and patients with chronic liver disease. We employed iCCA precision-cut tumour slices (PCTS) to characterise the patient-specific ECM and investigate the effects of a cyclophilin inhibitor (rencofilstat, currently in Phase 2 clinical evaluation for metabolic dysfunction-associated steatohepatitis) in combination with nivolumab.

**Method:** iCCA-PCTS from 4 patients were cultured for 7 days and exposed to rencofilstat (R) with/without nivolumab (N) and compared to untreated or standard of care (gem-cis + durvalumab). Tumour viability, proliferation, and stromal collagen analysis were studied by HandE, TUNEL, Ki-67 and Sirius red staining. A comprehensive analysis of different features of ECM patterns was done using TWOMBLI.

**Results:** The original patient-specific fibro-desmoplastic stroma was preserved in PCTS over culture and the analysis on Sirius red stained tissue using TWOMBLI highlighted characteristic features of the TME. Strikingly, TWOMBLI analysis performed on PCTS treated with R+N showed remarkable changes compared to untreated PCTS, including reduced fibre alignment and high-density matrix, increased lacunarity and N points/branches. Our findings demonstrate a significant ECM remodelling, hinting at potential therapeutic benefits. Although the short duration of the ex vivo treatment, including standard of care, did not yield significant changes in tumour death or proliferation, it notably offers insights into potential therapeutic avenues.

**Conclusion:** To conclude, we demonstrated the utility of iCCA PCTS for characterising patient-specific ECM and assessing combination therapies targeting the TME. In addition, PCTS represent a unique model to identify alternative surrogate measures of efficacy in vitro, that could be validated in patient cohorts. Finally, we show that R+N can favourably alter the TME in iCCA, suggesting that targeting both a dysfunctional ECM and exhausted tumour immune population to improve anti-cancer immune cell infiltration and function may be a promising avenue, deserving further investigation.

**Figure:**


Performed in Operating Theatre
Primary Liver Cancer
Surgical tissue resection
UW solution
Tissue specimen
5mm x 250µm
Slicing PCTS
Rencofilstat
Nivolumab
Patient recruitment
Histology: H&E & Picrosirius Red staining
ECM: TWOMBLI
Viability: TUNEL staining
Proliferation: Ki67 IF staining
Assessment of Efficacy
37°c
5% CO2
70 rpm
Culture
up to 8 days

# PO3-14
# The awareness of the link between liver cancer and hepatitis as a motivation for action-results of a globally representative survey

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**Background and aims:** Viral hepatitis is the most common risk factor for liver cancer. Chronic viral hepatitis can lead to hepatocellular carcinoma, which accounts for 80% of liver cancer cases and is the third most common cause of cancer deaths worldwide. Despite this, few studies have been conducted on the public awareness of hepatitis' connection to liver cancer. To gain a greater understanding of public awareness on the link between liver cancer and hepatitis, the World Hepatitis Alliance (WHA) undertook a globally representative survey in nine countries.

**Method:** The WHA developed a survey, consisting of five multiple choice questions to assess awareness of the link between viral hepatitis and liver cancer and if that knowledge affects their likelihood to be tested or vaccinated for hepatitis. The survey was fielded in nine countries that were selected based on geographic representation and their rates of liver cancer. The Survey Monkey platform distributed the survey to the public between 30 June to 4 July 2023.

**Results:** 569 participants responded to the globally representative survey from Argentina, Germany, Ghana, Hong Kong, India, Nigeria, South Korea, Vietnam, and the USA. The survey found that nearly half (42%) of people are unaware that one of the leading causes of liver cancer is viral hepatitis. Nearly three quarters (74%) of the participants responded that knowing hepatitis causes liver cancer means they are more likely to get tested and over four fifths (82%) responded they are more likely to get vaccinated.

**Conclusion:** The survey indicated that a significant percentage of people are unaware of the link between hepatitis and liver cancer. If people are aware of the connection, they felt more likely to get tested for hepatitis and to be vaccinated against hepatitis B. This demonstrates why it is critical to raise awareness that hepatitis is one of the leading causes of liver cancer to accelerate progress towards the WHO's goal to eliminate hepatitis by 2030. Increased vaccination for hepatitis B and treatment for hepatitis B and C can reduce the global incidence of liver cancer. Health systems should integrate hepatitis services into their cancer prevention programs to decrease cancer mortality and support hepatitis elimination.

**Figure:**

| Question | Responded 'Yes' | Responded 'No' | Responded 'Unsure' | Responded 'Comments' | Skipped Question | Total responses |
|---|---|---|---|---|---|---|
| **Did you know that one of the leading causes of liver cancer is viral hepatitis?** | 57.71% (322 responses) | 42.29% (236 responses) | Not an option | Not an option | 11 | 558 |
| **Are you more likely to get tested for hepatitis because you know hepatitis causes liver cancer?** | 72.91% (401 responses) | 24.18% (133 responses) | 2.91% (16 responses) | Not an option | 19 | 550 |
| **Are you more likely to get vaccinated for hepatitis B because you know it also protects against liver cancer?** | 80.69% (443 responses) | 16.94% (93 responses) | 2.00% (11 responses) | 0.36% (2 responses) | 20 | 549 |

# PO3-16
# Therapeutic potential of HuR inhibition in chronic liver disease and hepatocellular carcinoma

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**Aims:** Human antigen R (HuR) and tristetraprolin (TTP) are key RNA-binding proteins, involved in the regulation of the stability and translation of a wide range of transcripts involved in metabolic, inflammatory, and cancer-related processes (e.g., TNFα, MYC). Previous work and preliminary data indicate that both HuR and TTP alteration contribute to the development of hepatocellular carcinoma (HCC), but also chronic liver diseases fostering hepatocarcinogenesis, such as non-alcoholic steatohepatitis (NASH). The role of HuR and TTP in these diseases is still poorly known. Herein, our goal is to further characterize the role of HuR and TTP and evaluate the therapeutic potential of HuR inhibition in these diseases.

**Method:** The expression of HuR and TTP were measured in liver tissues from patients or mouse models with hepatic steatosis, NASH, HCC and intrahepatic cholangiocarcinoma (ICC). The impact of HuR silencing (siRNA) or inhibition with pharmacologic inhibitors (CMLD-2 and MS-444) on cancer-related processes, hepatic metabolism and fibrosis were investigated in a panel of hepatic cancer cell lines but also in primary hepatocytes and precision-cut liver slices (PCLS). Finally, a proteomic analysis was performed on hepatic cancer cells to characterize the impact of HuR inhibition, and identify potential targets.

**Results:** HuR expression is upregulated in both HCC and ICC, as compared to non-tumoral tissues. Interestingly, this effect occurs concomitantly with a decrease of TTP expression in liver cancers. Our analyses show a strong heterogeneity in HuR localization, with some patients displaying a strict nuclear localization, while for others; a nuclear and cytoplasmic localization was observed. Inhibition of HuR expression/activity (CMLD-2 and MS-444) strongly hinders hepatic cancer cells proliferation and viability. For MS-444 but neither for CMLD-2 nor HuR silencing, an accumulation of the cells in prophase was observed, thus suggesting some HuR-independent effects. Our proteomic analysis corroborates this phenotype and highlights the alteration of several cancer-related proteins (e.g., NONO, NUMA1, TRIM33). In primary hepatocytes and PCLS, HuR inhibitors promote lipid accumulation, thus suggesting cautions regarding the use of these inhibitors for therapeutic purpose.

**Conclusion:** HuR expression is strongly increased in hepatic cancers and contributes to the proliferation and survival of HCC cells. Targeting HuR with pharmacologic inhibitors may therefore represent an appealing therapeutic approach, as suggested in many cancers. Herein, we have highlighted these anti-cancerous properties in HCC but we have also shown that HuR inhibition importantly alters hepatic lipid metabolism. Whether this effect is HuR-dependent remains to be demonstrated and future work on mouse models is required to characterize the beneficial and detrimental effects of HuR targeting.

# PO3-17-YI
# Targeting sirtuins with novel indole derivatives: implications for anticancer activity in hepatocellular carcinoma

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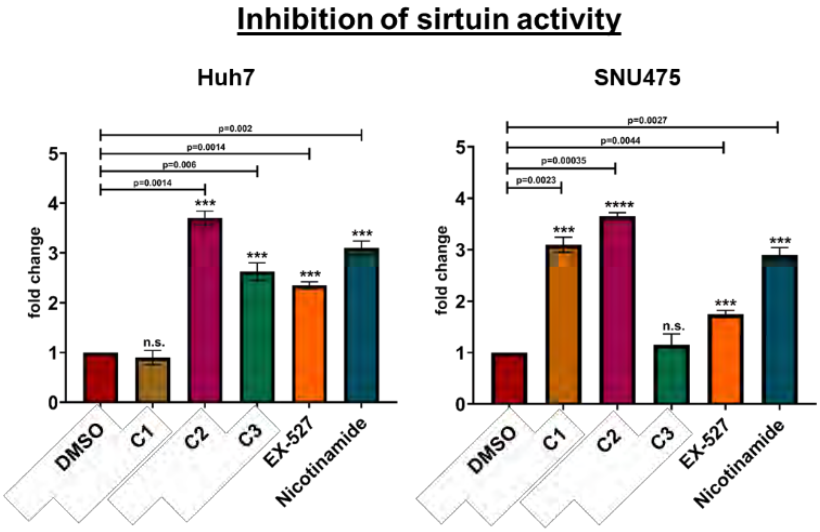
**Background and aims:** Hepatocellular carcinoma (HCC) is the major subtype of primary liver cancer and is the fastest-rising cause of cancer-related deaths globally. The complex pathogenesis of HCC involves diverse genomic alterations, contributing to tumorigenesis and poor overall survival rates. Among the dysregulated protein families in HCC, sirtuins, particularly SIRT1 is overexpressed and promotes cell survival and metastasis. This study addresses the potential anticancer treatment strategy of inhibiting SIRT1 using novel indole derivative compounds. The main objective of this study is to assess the anticancer activity of indole derivative compounds and to identify their potential to impede sirtuin activity in HCC cell lines.

**Method:** Sulforhodamine B (SRB) assay was performed to screen the compounds on cancer cell lines. An artificial intelligence (AI) based drug-target interaction (DTI) prediction method called DEEPScreen was utilized to evaluate the possible interaction of selected compounds with the sirtuin family proteins. Molecular docking studies and sirtuin activity assays were conducted to validate DTI predictions. Cellular mechanisms affected by the compounds in HCC cells were assessed by propidium iodide staining for cell cycle assay and AnnexinV/PI staining for apoptosis assay using flow cytometry. Immunofluorescence (IF) staining with Hoescht 33258 was done to identify apoptotic nuclear blebbing in HCC cell lines.

**Results:** Among 28 synthesized indole derivatives, 15 had IC50 values less than 10 μM, indicating significant inhibitory activity in Huh7 cells. Among them, 4 were selected based on their structure-activity relationship. According to DEEPScreen DTI predictions, the selected compounds had active drug-target interactions with SIRT1 or SIRT2 with performance scores higher than 80%, but not with SIRT3. Docking studies have shown that three compounds (C1, C2 and C3) were most potent in terms of their interaction with SIRT1. Among all selected compounds, the estimated free binding energy of compound 2 (C2) was lowest (-11.32 kcal/mol) and caused the highest sirtuin activity inhibition in HCC cell lines, outperforming well-known sirtuin inhibitors EX-527 and nicotinomide. Furthermore, C2 significantly induced G1 arrest and apoptotic cell death in HCC cell lines after 24 hours of treatment.

**Conclusion:** C2 is a potent anticancer agent inhibiting sirtuin activity in HCC cell lines. The results of this study highlight the promising role of indole derivative compounds as effective sirtuin inhibitors in anticancer interventions.

**Figure:**


Inhibition of sirtuin activity
Huh7
SNU475
fold change
p=0.002
p=0.0014
p=0.006
p=0.0014
p=0.0027
p=0.0044
p=0.00035
p=0.0023
***
****
n.s.
DMSO
C1
C2
C3
EX-527
Nicotinamide

## PO3-18
## Brg1 suppress DEN-induced hepatocellular carcinogenesis in mice

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**Background and aims:** Hepatocellular carcinoma (HCC) is the most prevalent primary liver cancer. Mutations of the SWItch/Sucrose NonFermentable (SWI/SNF) chromatin remodeling complex are commonly found in multiple tumors. Brahma-related gene 1 (Brg1) is a key member of the SWI/SNF family of proteins. However, the role of Brg1 in liver tumorigenesis and hepatocarcinogenesis remains unclear.

**Method:** Liver tumors were induced in wild-type (Control) and hepatocyte-specific Brg1 knock out mice (Brg1 KO) by intraperitoneal diethylnitrosamine (DEN) injection. Tumorigenesis was analyzed after 11 months and molecular analyses were performed at different stages of tumorigenesis.

**Results:** We showed that Brg1 expression is increased in tumor tissue compared to non-tumor tissue in DEN-induced HCC in wildtype mice. Liver tumor incidence, tumor numbers and tumor size were strongly increased in DEN-injected Brg1 KO mice compared to DEN-injected Control group, suggesting that Brg1 knockout contributes to tumor initiation in DEN-injected mice model. Besides, DEN-injected Brg1 KO mice had *shorter survival* compared to DEN-injected Control group.

**Conclusion:** Our data reveal that deletion of Brg1 in hepatocytes promotes hepatocarcinogenesis in DEN-induced mice.

## PO4-01-YI
## Targeting endoplasmic reticulum-stress pathways : AMG-PERK and TUDCA inhibitors as potential repressors for hepatocellular carcinoma progression

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**Background and aims:** Several pathways and underlying mechanisms are suggested to promote the development of hepatocellular carcinoma (HCC). One potential driver of HCC progression is the activation of endoplasmic reticulum (ER) stress pathways. This phenomenon occurs when ER homeostasis is impaired by various physiological, pathological or environmental conditions. This stress is characterized by a cellular process resulting from a prolonged need for protein synthesis that exceeds the capacity of the ER to ensure correct protein folding. The unfolded protein response is triggered by the accumulation of unfolded and misfolded proteins, with the aim of preserving ER function for cell survival or activating cell apoptosis. Due to the liver's synthesizing and purifying role, it is particularly exposed to the risk of ER stress. However, its role in HCC is not yet well defined. The aim is to assess whether different inhibitors of ER stress have influenced the progression of HCC.

**Method:** A chemically induced mouse model (5-week-old male Sv129) for HCC was used and mice were treated twice per week with the ER-stress inhibitors TUDCA and/or AMG-PERK for 3 weeks, after the occurrence of tumors. Liver samples were taken after 25 weeks for histological and molecular biology analyses.

**Results:** Preliminary results revealed that treatments with ER stress inhibitors significantly decreased tumor numbers. HandE staining exposed an increase in HCC mice's tumor burden, while being significantly reduced in all groups treated with ER stress inhibitors. In HCC mice, the percentage of liver tissue nuclei and cell count amplified while it significantly declined in all treatment groups with TUDCA and/or AMG-PERK. High nuclei levels could be an indicator of increased malignancy as it may influence tumor microenvironment by promoting metastasis. Liver fibrosis was quantified by Sirius Red staining, detecting increased collagen deposition in HCC mice. Mice treated with ER-stress inhibitors showed a reduction in collagen levels. Increased GOLPH2 protein levels were previously reported in HCC patients, thus indicating its potential contribution to HCC development, highlighting its relevance as an early diagnostic biomarker for HCC. Cell proliferation marker PCNA was highly expressed in HCC mice. Mice treated with AMG-PERK and/or TUDCA, presented downregulation in ER-stress biomarkers expression such as Hspa5, ATF4, Edem1, Herp, PERK, ATF6 and CHOP. Cell proliferation (PCNA, Ki67) and fibrosis (collagen) markers were decreased. Inflammation biomarkers (IL-6, CD40L, Araginase, TNF), were also downregulated

**Conclusion:** By using an in vivo model known for its similarity to human HCC, we show that using an ER-stress inhibitor can decrease the HCC progression. The long-term impact of our study could open the possibility of ER-stress inhibitors as supporting treatments to chemotherapy for HCC-patients.

# PO4-03-YI
# Development of microenvironment-dependent hepatocellular carcinoma and colorectal liver metastasis spheroid culture models

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**Background and aims:** Liver cancer is a leading cause of cancer-related deaths, with a significant portion of cancer metastases occurring in the liver. One of the reasons for failure of therapeutic compounds in clinical trials is the lack of relevant preclinical *in vitro* models. Both 2D and 3D culture models consisting solely of (cancer) cell lines are often used, although these models cannot accurately recapitulate the *in vivo* microenvironment. We aim to develop clinically more representative 3D *in vitro* culture models for hepatocellular carcinoma (HCC) and colorectal liver metastasis (CRLM) in which we can investigate cell communication between liver-resident cells and cancer cells.

**Method:** Hepatic stellate cells (HSCs), Kupffer cells, liver sinusoidal endothelial cells and hepatocytes were isolated from mouse livers using *in situ* perfusion and subsequent flow cytometry and density gradient techniques. Primary cells were allowed to aggregate with either 10 mCherry positive mouse hepatoma (Hepa1-6) or mouse colon adenocarcinoma (MC-38) cells and seeded in cell-repellent plates to generate scaffold-free spheroids. The spheroids are maintained in serum-free medium. Fluorescence microscopy was used to determine cancer cell proliferation. Cell viability assays, picrosirius stainings and qPCR analysis for HSC activation markers were carried out on day 2 and/or day 7.

**Results:** Over a period of 7 days, the 10 cancer cells grow rapidly inside the liver spheroid as demonstrated by the increase in mCherry positive cells. In contrast, when cancer cells are seeded alone as spheroids in the same serum-free medium, the cells fail to thrive as evidenced by increased cell death and reduced cell growth. Importantly, the addition of cancer cells appears to induce a fibrotic response in the spheroids as shown by increased collagen deposition and increased mRNA levels of HSC activation markers in the spheroids.

**Conclusion:** We have developed new relevant models to study HCC and CRLM. We could demonstrate that in these spheroid cultures, the liver microenvironment is essential for the support of liver cancer cell growth. Furthermore, we show that these cultures have the potential to mimic HSC to cancer associated fibroblast transdifferentiation. In the future, this model could be used as a screening tool for potential drug candidates before moving to *in vivo* models. This could greatly decrease the number of mice used in preclinical research and enhance the predictability of preclinical screens.

**Figure:**

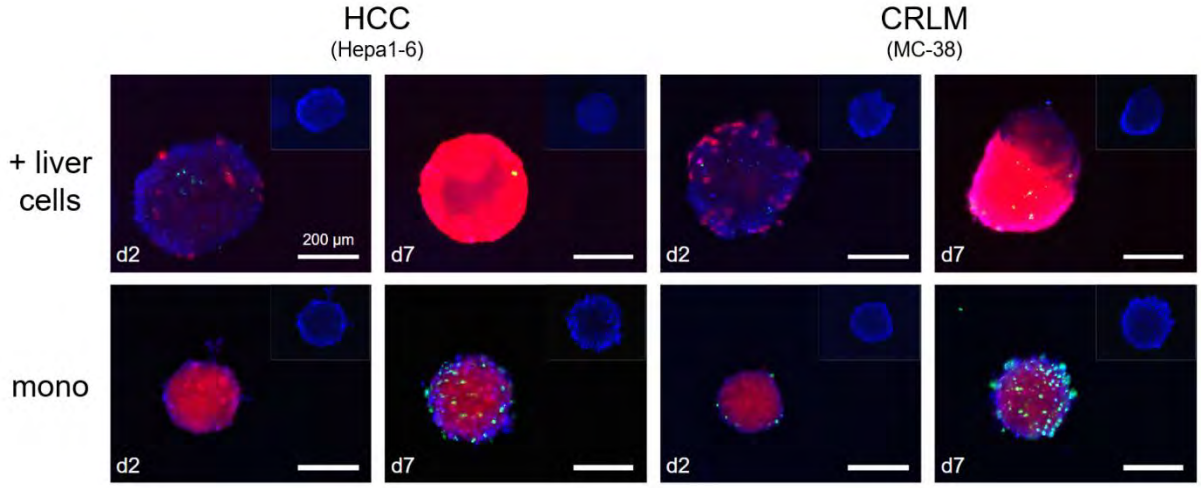


**Figure 1. Liver cancer cells rely on the liver microenvironment for their survival.** Cell viability staining of HCC cells (left) and CRLM cells (right) cultured with (top) and without (bottom) primary liver cells in 0% FBS on day 2 and day 7. Scale bar represents 200 µm. Fluorescent images of cancer cells (mCherry), dead cells (NucDead) and all cells (Hoechst) were taken using an EVOS M7000 microscope.

## PO4-04-YI

# Inhibiting the endoplasmic reticulum stress response enhances the effect of doxorubicin by altering the lipid metabolism of liver cancer cells

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**Background and aims:** Hepatocellular carcinoma (HCC) is considered a poor responder to chemotherapeutic treatments. One contributing factor to the overall pharmacodynamics is the activation of endoplasmic reticulum (ER) stress pathways. This is a cellular stress mechanism that becomes activated when the cell's need for protein synthesis surpasses the ER's capacity to maintain accurate protein folding, and has been implicated in creating drug-resistance in several solid tumors. Aim: to identify the role of ER-stress and lipid metabolism in mediating drug response in HCC.

**Method:** Employing a chemically-induced in vivo mouse model for HCC, we administered the ER stress inhibitor 4μ8C and/or DOX twice weekly for three weeks post-tumor initiation. Histological analyses were performed alongside comprehensive molecular biology and lipidomics assessments of isolated liver samples. In vitro models including HCC cells, spheroids, and patient-derived liver organoids were subjected to 4μ8C and/or DOX, enabling us to assess their effects on cellular viability, lipid metabolism, and oxygen consumption rate.

**Results:** We reveal a pivotal synergy between ER stress modulation and drug response in HCC. The inhibition of ER stress using 4μ8C not only enhances the cytotoxic effect of DOX but also significantly reduces cellular lipid metabolism. This intricate interplay culminates in the deprivation of energy reserves essential for the sustenance of tumor cells.

**Conclusion:** Our study merges the realms of lipid metabolism and ER stress modulation in HCC. This novel approach not only deepens our understanding of the disease but also uncovers a promising avenue for therapeutic innovation. The long-term impact of our study could open the possibility of ER-stress inhibitors and/or lipase inhibitors as adjuvant treatments for HCC-patients.

**Figure:**

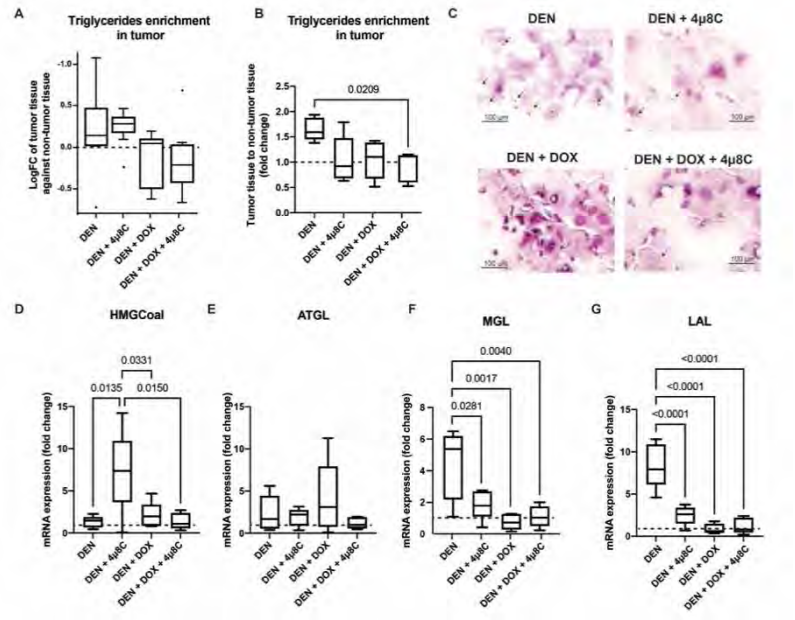


Figure 1. Treatment with DOX and 4μ8C reduces triglyceride content of liver tumors and alters lipid metabolism. (A) Triglyceride content of tumors measured through liquid chromatography-high resolution mass spectrometry and (B) *via* a commercial fluorimetric triglyceride assay. (C) Representative pictures of Oil-Red-O staining. mRNA expression of (D) HMG-CoA-reductase (E) ATGL, (F) MGL and (LAL). N = 5 mice per group. Fold change is compared to matched non-tumoral tissue, marked as dashed line in graphs.

# PO4-08
# TM4SF5-NCOA3-PTEN linkage for albumin uptake and bioenergetics during HCC progression

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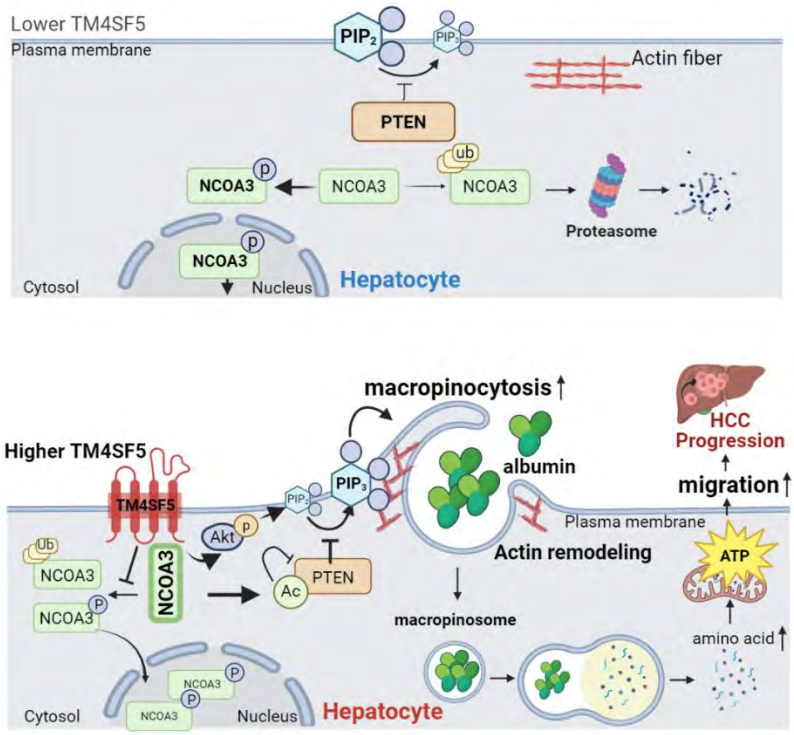
**Background and aims:** Transmembrane 4 L six family member 5 (TM4SF5) is involved in the development and progression of hepatocellular carcinoma (HCC) in an inflammatory-metabolic environment. Although TM4SF5 promotes cellular migration and invasion, it is unclear how the metabolic environment affects metastatic potential.

**Method:** Here, we explored how TM4SF5 affects uptake of the major serum component albumin, bioenergetics, and cellular migration using TM4SF5 knock-out or reintroduced hepatocyte and animal systems.

**Results:** Serum deprivation of hepatocytes led to differential formation of filipodia-like processes depending on TM4SF5 expression. Albumin replenishment (0.36 g/dL, ten times lower than the normal human serum level) changed these processes and ruffling via phosphatidylinositol (3, 4, 5)-trisphosphate ($PIP_3$) actions on plasma membranes, allowing macropinocytosis-mediated albumin uptake. Interestingly, unlike control TM4SF5 knock-out cells, macropinocytosis of TM4SF5 wild-type cells was sufficient with albumin replenishment alone, whereas glucose replenishment to A132V mutant cells was a prerequisite for efficient albumin uptake, presumably guided by activity of mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK). During macropinocytosis, nuclear receptor coactivator 3 (NCOA3) was cytosolically stabilized via association with TM4SF5 and phosphatase and tensin homolog deleted on chromosome 10 (PTEN), thus possibly rendering higher $PIP_3$ levels. Such TM4SF5-dependent albumin uptake was followed by ATP-linked respiration and cellular migration depending on NCOA3 expression and albumin catabolism. Furthermore, cancerous liver tissue in orthotopic mice fed a high protein diet and human liver cancer tissue showed TM4SF5-dependent macropinocytosis and NCOA3-correlated metastatic features compared with mice fed a normal chow diet or human non-tumour regions.

**Conclusion:** These observations indicate that high serum albumin availability to TM4SF5-positive HCC could support multifocality and intrahepatic metastasis, which may provide insights into clinical observations of multiple small tumour nodules surrounded by high serum albumin.

**Figure:**


Lower TM4SF5
Plasma membrane
PIP2
PIP3
Actin fiber
PTEN
ub
NCOA3
P
NCOA3
NCOA3
NCOA3
Proteasome
P
NCOA3
Cytosol
Nucleus
Hepatocyte
macropinocytosis
albumin
HCC
Progression
migration
Higher TM4SF5
TM4SF5
PIP3
PIP2
Plasma membrane
Akt
P
Actin remodeling
ATP
Ub
NCOA3
NCOA3
P
NCOA3
Ac
PTEN
macropinosome
amino acid
NCOA3
P
NCOA3
Cytosol
Nucleus
Hepatocyte

## PO4-09-YI
# MiR-22 downregulation activates HIF-1A pathway and induces tumor progression, metabolic reprogramming and sorafenib resistance in hepatocellular carcinoma

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**Background and aims:** Hepatocellular carcinoma (HCC) is the third cause of cancer-related mortality worldwide. Around 40% of patients are diagnosed at advanced stages when only systemic therapies are available. Although immunotherapy has revolutionized HCC treatment, not all patients are eligible, in particular metabolic-driven HCCs or certain genetic backgrounds; in these cases, the TKIs sorafenib and lenvatinib represent the first choice. Acquired drug resistance is a major cause of treatment failure, and identifying new therapeutic combinations remains an open issue. MicroRNAs (miRs) contribute to tumorigenesis and metabolic shift of hepatocytes. MiR-22 is commonly downregulated in cancers, including HCC, being considered as a tumor suppressor miR. The aim of this study is to investigate miR-22 contribution to HCC tumorigenesis and sorafenib response, focusing on its possible role in metabolic reprogramming.

**Method:** MiR-22 levels were analyzed by Real Time PCR in DEN-HCC rats and HCC patients. Proliferation, apoptosis, migration, and spheroid formation assays demonstrated miR-22 influence on HCC phenotype *in vitro*, whereas a xenograft mouse model was used to determine miR-22 role on HCC tumorigenesis *in vivo*. Functional analysis elucidated the relationship between HIF-1A pathway activation and miR-22 in different settings. Metabolic assays evaluated miR-22 involvement in aerobic glycolysis and ROS production in HCC cells.

**Results:** We reported the downregulation of miR-22 in human and rat HCC specimens compared to surrounding livers, as well as its association with microvascular invasion, tumor grade, and a worse overall survival. *In vitro* assays in HCC cells stably overexpressing or silenced for miR-22 revealed that miR-22 inhibits both 2D/3D cell growth and cell migration. Regarding *in vivo* tumorigenesis, miR-22-silenced cells gave rise to bigger tumors whereas miR-22-overexpressing cells resulted in smaller tumors compared to controls. Lower miR-22 levels associated with sorafenib resistance in the rat model. Activation of HIF-1A was detected in miR-22-silenced HCC cells and spheroids, together with a cell growth advantage in hypoxic conditions and in the presence of sorafenib. As concerns cell metabolism, miR-22 silencing promoted a metabolic shift towards a glycolytic phenotype, as showed by a reduction in oxygen consumption rate and an increase in glucose consumption, extracellular lactate levels, and intracellular lipids.

**Conclusion:** Low miR-22 levels favor HCC tumorigenesis and metabolic reprogramming, associating with a poor prognosis. Interestingly, miR-22 has an impact on sorafenib sensitivity, possibly via HIF-1A stabilization and glycolytic metabolism. If confirmed in animal models, the use of miR-22 mimics in combination with sorafenib deserves attention for the treatment of HCC patients being ineligible for immunotherapy.

# PO4-10
# Histological and molecular characterization of GAN diet-induced obese mouse model of advanced fibrosing MASH with progression to HCC

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**Background and aims:** Metabolic dysfunction-associated steatohepatitis (MASH) is a leading cause of liver cirrhosis and hepatocellular carcinoma (MASH-HCC). However, the molecular alterations leading to onset of MASH-HCC are unclear. The present study aimed to evaluate disease progression in the translational GAN diet-induced obese (DIO) mouse model of advanced fibrosing MASH-HCC (GAN DIO-MASH-HCC mice).

**Method:** Male C57BL/6J mice were fed the GAN diet high in fat, fructose and cholesterol for 38 78 weeks (n = 15 per group). Mice fed chow for 48-68 weeks (n = 15) served as healthy controls. Terminal end points included AI-assisted histopathological scoring and histomorphometrics, flow cytometry, tumor classification by an expert clinical histopathologist and bulk RNAseq. Single sample gene set enrichment analysis (ssGSEA) and digital cytometry (xCell, EcoTyper) was performed. Transcriptional profiles of murine MASH and MASH-HCC were compared to human hepatic transcriptomes from healthy controls to MASH (n = 57, GSE126848) and MASH-HCC (n = 45, GSE193084).

**Results:** GAN DIO-MASH-HCC mice demonstrated NASH (NAFLD Activity Score ≥5), progressive fibrosis (all mice demonstrating F3 at ≥60 weeks) and HCC burden consistent with human MASH-HCC. Liver inflammation was characterized by expansions in inflammatory M1 macrophages, Kupffer cells, dendritic cells and CD8+ T-cells. Hepatic neoplasms were detected from 48 weeks with progressive cohort penetrance, resulting in 100% incidence at 72 weeks of GAN diet feeding. 70% of tumors presented histological features of poor prognostic HCC; 30% were classified as hyperplastic nodules. Transcriptional analysis associated murine MASH-HCC tumors with S1 Hoshida signature, activation of immune responses, KRAS, p53 and Wnt/β-catenin signalling pathways. Digital cytometry assigned 50% of murine MASH-HCC tumors carcinoma ecotypes associated with short survival (CE1, CE2), while 30% of tumors presented an ecotype associated with positive response to immune therapy (CE9).

**Conclusion:** The GAN-DIO-MASH-HCC model spontaneously develops HCC on the background of progressive advanced fibrosis. The HCC molecular signature recapitulates poor prognostic human MASH-HCC and immune microenvironment. The translational GAN DIO-MASH-HCC mouse model is highly applicable for profiling novel drug therapies targeting NASH-HCC, including first-line immune checkpoint inhibitor therapies.

# PO4-11
# Sensitization of cholangiocarcinoma to chemotherapy by β-caryophyllene oxide-induced BCRP inhibition

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**Background and aims:** Cholangiocarcinoma (CCA) is an aggressive liver cancer with poor response to available drugs, partly due to its marked multidrug resistance (MDR) phenotype, which is the result of several mechanisms of chemoresistance (MOCs) either endogenous or that arise in response to administered anti-tumor agents. Some MOCs lead to a decrease in drug concentration within the cellular compartment carried out, among others, by ATP-binding cassette (ABC) proteins. Consistently, using inhibitors of ABC pumps is a promising strategy against highly resistant tumors. In this regard, beta-caryophyllene oxide (CRYO), a natural sesquiterpene present in many plants worldwide, has been extensively studied for its potent analgesic, anti-inflammatory, and genoprotective effects. Moreover, we have demonstrated that, used at sub-toxic doses, CRYO can enhance the response of hepatocellular carcinoma (HCC) cells to sorafenib due to its ability to inhibit several ABC proteins. The aim of this work was to evaluate the ability of CRYO to enhance the response of CCA cells to first- and second-line drugs used against this cancer by blocking ABC-mediated drug export, with a particular focus on BCRP.

**Methods:** Transcriptomic data of CCA patients included in the "The Cancer Genome Atlas" (TCGA) were used to analyze BCRP expression in the tumor and adjacent non-tumor tissue. To evaluate the ability of CRYO to inhibit BCRP, loading and efflux assays of mitoxantrone, a fluorescent substrate of BCRP, were performed on EGI-1 and TFK-1 cells. To validate the effects of CRYO on BCRP pump activity, a cellular model of BCRP overexpression in Chinese hamster ovary (CHO) cells was used. BCRP expression was determined by RT-qPCR and western blot. The effect on cell viability of CRYO combined with cisplatin, gemcitabine, mitoxantrone, SN-38, sorafenib, 5-fluorouracil, and oxaliplatin in BCRP-overexpressed cells was evaluated by MTT-formazan assay.

**Results:** BCRP expression was relatively preserved in intra (iCCA), and extra (eCCA) hepatic CCA, and cell lines derived from CCA (EGI-1 and TFK-1). CRYO enhanced mitoxantrone load into BCRP-expressing CHO cells by inhibiting its efflux to a greater extent than induced by FTC, a potent and specific BCRP inhibitor. Additionally, CRYO significantly potentiated the effect of first- and second-line drugs against CCA, such as cisplatin, mitoxantrone, SN-38, and sorafenib, and had a moderate impact when combined with gemcitabine, 5-FU, and oxaliplatin.

**Conclusion:** At non-toxic concentrations, CRYO enhances the sensitivity of CCA cells to anti-tumor drugs by inhibiting ABC proteins, such as BCRP, suggesting its potential usefulness in combination treatments against CCA.

*This study is based upon work from ENSCCA and COST Action Precision-BTC-network (CA22125), supported by COST (European Cooperation in Science and Technology).*

# PO4-12-YI
# In silico electrophysiology investigation unveils cardiotoxicity Induced by Tecentric, a crucial therapeutic agent for hepatocellular carcinoma, through sodium current inhibition

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**Background and aims:** Tecentric, a pivotal therapeutic agent for urothelial carcinoma, small cell lung cancer, and hepatocellular carcinoma, has been associated with sinus bradycardia, impacting patients' quality of life. Although cardiotoxicity is under clinical scrutiny, this study aims to elucidate how Tecentric concentrations modulate cardiac electrophysiological properties.

**Method:** Employing a sinoatrial node (SAN) electrophysiological setup, we focused on the inward rectifier ion channels, sodium channels (Nav1.5), potassium channels, calcium channels, and calcium diffusion mechanisms Tecentric concentrations ranging from 0.1 µmol/L to 10 µmol/L were applied for 200 ms to gauge their influence on Nav1.5 conductance. Both current-clamp and voltage-clamp protocols were utilized to record electrophysiological activities.

**Results:** Varying current stimulus (Istim) elicited action potentials (AP) in the SA node. Tecentric exhibited concentration-dependent effects on Nav1.5, evident in the current-voltage (I-V) curve under voltage clamp. The inward current progressively decreased, reaching 26% of the control value at 10 µmol/L. The I-V curve shifted positively by 20%, and the half-activation potential increased by 28%. Incorporating the altered inward current into the whole-cell model revealed prolonged repolarization and reduced firing frequency at 10 µmol/L Atezolizumab. Figure illustrations display AP patterns for control and Tecentric injection.

**Conclusion:** Our findings indicate that higher concentrations of Tecentric suppress Nav1.5 current, leading to a reduction in the frequency of spontaneous AP firing. This underscores the importance of controlling Tecentric dosage to mitigate potential cardiac toxicity. Further exploration through clinical trials is imperative to unravel its subcellular mechanisms.

## PO4-17

# The role of adenosine monophosphate-activated protein kinase pathway in sinusoidal endothelial dysfunction during the pathogenesis of metabolic dysfunction-associated steatohepatitis-driven hepatocellular carcinoma

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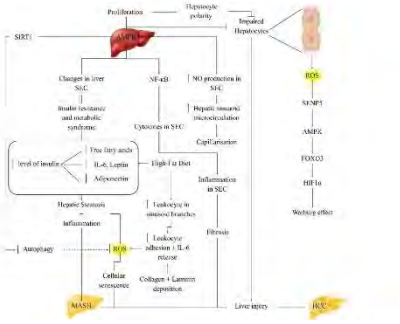
**Background and aims:** Hepatocellular carcinoma (HCC) is a primary liver cancer with a high mortality rate, often associated with chronic liver diseases such as metabolic dysfunction-associated steatohepatitis (MASH). The adenosine monophosphate-activated protein kinase (AMPK) pathway plays a crucial role in regulating cellular energy homeostasis and has been implicated in HCC. Nevertheless, the specific role of AMPK pathway in sinusoidal endothelial cells (SECs) dysfunction during MASH in HCC pathogenesis remains unclear. We aim to theorize the role of AMPK in SECs dysfunction in HCC pathogenesis.

**Method:** A search of the literature was conducted to identify relevant studies investigating the theorized objectives of the study. Studies exploring the molecular mechanisms, signaling pathways, and potential therapeutic targets related to AMPK pathway alteration in MASH and HCC were included in this study.

**Results:** The study's results indicate that dysregulation of the AMPK pathway plays a significant role in the dysfunction of SECs during MASH, contributing to the development and progression of HCC. In HCC, dysregulated energy metabolism, characterized by enhanced glycolysis (Warburg effect), provides growth advantages in the tumor microenvironment. Activation of AMPK in HCC counteracts this metabolic rewiring by promoting oxidative metabolism, restricting tumor growth and benefiting healthy hepatocytes. Dysfunctional AMPK signaling is associated with increased angiogenesis, inflammation, and oxidative stress in the tumor microenvironment, promoting HCC growth and metastasis. Dysregulation of AMPK in SECs is linked to MASH-induced HCC pathogenesis through dysregulated fatty acid metabolism, ATP depletion, and subsequent activation of downstream pathways. AMPK dysregulation is observed in HCC tissues, with altered activation compared to non-tumor liver tissues, and AMPK deficiency could accelerates HCC formation in mice. AMPK also exerts tumor-suppressive effects by modulating cell cycle progression, inhibiting angiogenesis, and promoting apoptosis. Components of the AMPK pathway, such as liver kinase B1 (LKB1) and sirtuin 1 (SIRT1), further contribute to metabolic dysregulation in SECs. It can modulate cell cycle progression by inhibiting cell cycle regulators such as mammalian target of rapamycin (mTOR) and promoting the expression of p53 and p27, which leads to cell cycle arrest and apoptosis. Inhibition of the AMPK/mTOR pathway could possibly increase the radiosensitivity of hepatocellular carcinoma in mice.

**Conclusion:** Alteration of the AMPK pathway in SECs during MASH could be a possible critical pathway in HCC pathogenesis. Targeting this pathway could be important for HCC prevention and treatment in MASH patients. Further extensive precise research is required to understand the underlying mechanisms and develop therapeutic interventions.

**Figure:**


Proliferation
Hepatocyte polarity
Impaired Hepatocytes
SIRT1
AMPK
Changes in liver SEC
NF-κB
NO production in SEC
ROS
Insulin resistance and metabolic syndrome
Hepatic sinusoidal microcirculation
NEMP1
Cytokines in SEC
AMPK
Capillarization
FOXO3
Free fatty acids
level of insulin
IL-6, Leptin
Adiponectin
High-Fat Diet
HIF1α
Inflammation in SEC
Warburg effect
Hepatic Steatosis
Leukocyte in sinusoid branches
Inflammation
Fibrosis
Leukocyte adhesion / IL-6 release
Autophagy
ROS
Cellular senescence
Collagen I Laminin deposition
MASH
Liver injury
HCC

## PO4-18-YI

# A preliminary collection of healthy and tumoral patient-derived organoids for the screening of personalized therapies in hepatocellular carcinoma

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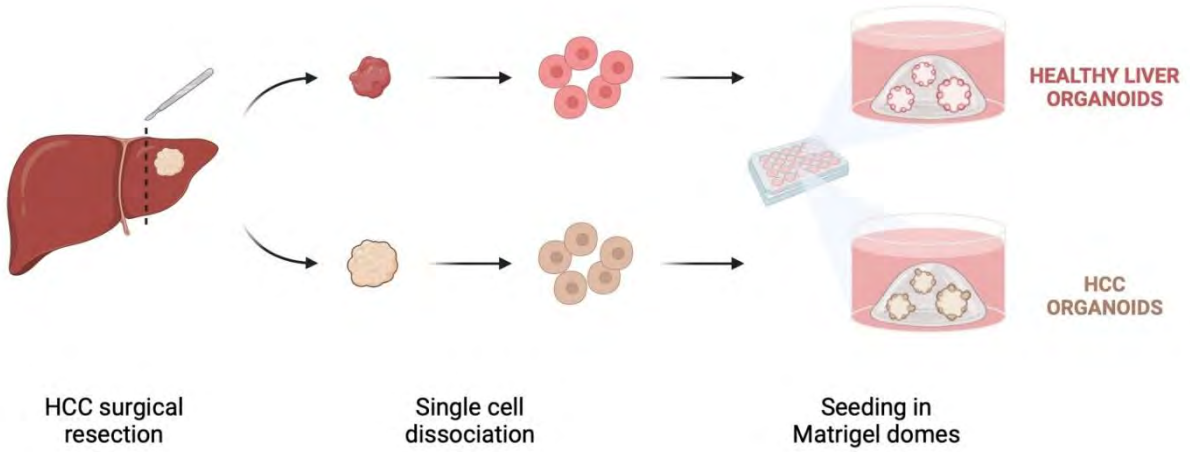
**Background and aims:** Hepatocellular carcinoma (HCC) is the main primary liver cancer and the third cause of cancer-related mortality worldwide. Due to high inter- and intra-individual heterogeneity, multiple risk factors and genomic contexts, advanced HCC is characterized by limited response to systemic treatments and early onset of drug resistance. Thus, there is an urgent need for new therapies and biomarkers, but a major obstacle to clinical translation has been the lack of appropriate preclinical tools. Patient-derived organoids (PDOs) may overcome this limitation, as they faithfully recapitulate the architecture, functions, and genetic background of the original tissue. The aim of this study is the generation of healthy and tumoral PDOs from HCC patients with different aetiologies, to be used for the screening of personalized therapies.

**Method:** PDOs were generated from HCC tumors and surrounding non-malignant tissues of patients undergoing liver resection at IRCCS Policlinico S. Orsola, Bologna. Tissues were processed by mechanical and enzymatic dissociation, seeded in Matrigel, and cultured with the specific Initiation, Growth, and Differentiation Media supplied by the HepatiCult™ Organoid Kit (StemCell Technologies). Total RNA from organoids was extracted and analysed by Real Time PCR. Drug treatment with sorafenib or lenvatinib was monitored with the live imaging system Incucyte and evaluated as organoids' area/darkness over time.

**Results:** We established several PDOs from HCC tumors with different aetiologies, as well as from their surrounding liver (SL) tissue. The success rate is 70% for SL (n = 7) and 60% for HCC (n = 10). The morphology of SL organoids is cystic, while HCC organoids differ among patients, with some appearing more like solid structures, others more cystic. Real Time PCR analysis revealed a reduced expression of stemness markers (AXIN2, SOX9) and increased expression of biliary/progenitor (CK19) and HCC-specific (GPC3, AFP) markers in HCC organoids compared to their normal counterpart. We induced differentiation in both SL and HCC organoids, as confirmed by the reduced expression of stemness markers (LGR5, CD133, SOX9) and increased expression of mature hepatocytes/epithelial markers (ALB, CYP3A4, EpCAM) compared to controls. A preliminary drug-sensitivity test on PDO HCC26 showed a strong response to sorafenib but no response to lenvatinib.

**Conclusion:** We successfully established a preliminary collection of healthy and HCC PDOs that retain the expression of markers distinctive of the tissue of origin. We observed a different sensitivity of HCC organoids to TKIs, supporting the use of PDOs as reliable personalized models for drug screenings. Further genetic and morphological characterization and expansion of the collection will be fundamental to obtain a comprehensive preclinical platform for the study of tailored treatments in HCC.

**Figure:**


HEALTHY LIVER ORGANOIDS
HCC ORGANOIDS
HCC surgical resection
Single cell dissociation
Seeding in Matrigel domes

## PO5-02
# Emerging role of oncogenic ß-Catenin in Exosome biogenesis as a driver of immune escape in hepatocellular carcinoma

Camille Dantzer[1], Justine Vaché[1], Aude Brunel[2], Isabelle Mahouche[1], Anne-Aurélie RAYMOND[1], Jean-William Dupuy[3], Mélina Pétrel[4], Paulette Bioulac-Sage[1], David Perrais[5], Nathalie DUGOT-SENANT[6], Mireille Verdier-Sage[2], Barbara Bessette[2], Clotilde Billottet[1], Violaine Moreau[1]
*[1]Univ. Bordeaux, INSERM, BRIC, U1312, Bordeaux, France*, *[2]Univ. Limoges, INSERM, CAPTuR, U1308, Limoges, France*, *[3]Plateforme Protéome, Univ. Bordeaux, Bordeaux Proteome, Bordeaux, France*, *[4]Bordeaux Imaging Center, Univ. Bordeaux, CNRS, INSERM, BIC, US4, UAR 3420, Bordeaux, France*, *[5]Univ. Bordeaux, CNRS, Interdisciplinary Institute for Neuroscience, IINS, Bordeaux, France*, *[6]Plateforme d'histologie, UMS005, TBMCore, Univ. Bordeaux, Bordeaux, France*

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**Background and aims:** Immune checkpoint inhibitors have produced encouraging results in patients with hepatocellular carcinoma (HCC). However, the majority of ß-catenin mutated HCC have been described as lacking immune infiltrates and resistant to immunotherapy. The mechanisms by which the oncogenic ß-catenin affects immune surveillance remain unclear. Herein, we addressed the role of ß-catenin in the regulation of the exosomal pathway and in the immune/cancer cell communication.

**Method:** New transcriptional targets of ß-catenin were identified using transcriptomic and proteomic analyses of allele specific knock-down in HepG2 cells. Gene expression was quantified using qRT-PCR and western-blot approaches. Exosome secretion was analyzed using nanoparticle tracking analysis and electron microscopy of conditioned media from cultured liver cancer cells. Immune cell infiltration into 3D spheroid models was evaluated by flow cytometry after co-culture with Peripheral Blood Mononuclear Cells. The expression of exosomal markers was assessed in human HCC samples using analysis of public transcriptomic data, and immunohistochemistry on 56 human HCC samples bearing or not *CTNNB1* mutations.

**Results:** Using transcriptional and proteomic analyses of the dual ß-catenin knockdown HepG2 model we published recently (Gest, Sena et al., 2023), we highlighted that mutated ß-catenin silencing enhanced significantly the expression of genes linked to the exosomal machinery. We showed that mutated ß-catenin represses *SDC4* and *RAB27A* gene expression in liver cancer cell lines and in HCC patient samples. Using nanoparticle tracking and a pH-sensitive reporter (CD63-pHluorin) allowing the visualization of MVB-PM fusion by live-cell imaging, we showed that activated ß-catenin represses exosome release. Then, we demonstrated in 3D spheroid models that activation of β-catenin promotes a decrease of immune cell infiltration through a default in exosome secretion. Furthermore, using two independent cohorts of HCC patients, we revealed that low *RAB27A* and *SDC4* expression is a feature of ß-catenin-mutated human HCCs.

**Conclusion:** Altogether our results provide new insights into the impact of β-catenin mutations on the liver tumor microenvironment. We revealed that oncogenic β-catenin repressed exosome secretion from tumor cells impacting crosstalk and infiltration of immune cells into tumors. These new findings may enable the development of new strategies to increase immunotherapy response.

## PO5-03-YI

# Targeting hepatocellular carcinoma: evaluation of vicinal diaryl isoxazole and pyrazole derivatives with implications for oxidative stress and senescence-mediated anti-tumor mechanisms

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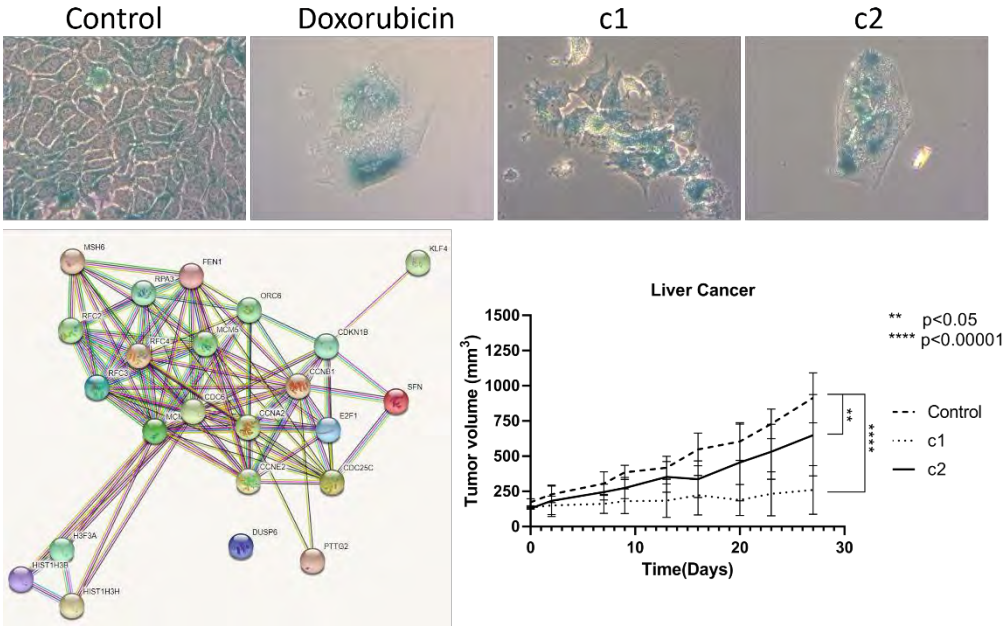
**Background and aims:** Hepatocellular carcinoma (HCC) is recognized as the predominant form of primary liver cancer and ranks as the third leading cause of global cancer-related fatalities. Anticipated to increase in the coming decade, the worldwide prevalence of HCC is driven by the escalating incidence of obesity and its associated fatty liver disease. Conventional chemotherapies encounter significant challenges in overcoming HCC resistance due to the hyperactivation of critical cell survival signaling pathways. Despite the limited efficacy of existing targeted agents, which extend patient survival by six months, the need to devise innovative therapeutics against HCC remains paramount. This study explores cytotoxic bioactivities exhibited by a novel vicinal diaryl isoxazole and pyrazole derivatives against HCC.

**Method:** Following the assessment of compound cytotoxicity using real-time cell electronic sensing (RT-CES), we investigated cell cycle progression, cell death, reactive oxygen species (ROS), and DNA damage through flow cytometry analysis. Transcriptome alterations were examined using a multiplex high-throughput NanoString nCounter Platform with a PanCancer Pathways panel comprising 770 cancer-related genes. Senescence-associated β-galactosidase activity and oxidative stress were assessed through immunostaining. Western blot analysis was employed to study pathways associated with cell cycle regulation, senescence, and oxidative stress. *In vivo*, the anti-cancer efficacy of the compounds was evaluated using a tumor xenograft assay.

**Results:** Among 60 compounds, two (c1 and c2) demonstrated high cytotoxicity against cells at low concentrations. Treatment with compounds c1 and c2 exhibited time- and dose-dependent growth inhibition. The transcriptomic analysis of the PanCancer Pathways panel revealed significant differentiation in the cell cycle pathway and associated genes. Morphological changes indicative of senescence, such as enlarged and multinucleated cells, were observed upon treatment with the compounds. This was attributed to series of biochemical events, including oxidative stress-associated DNA damage, senescence, cell cycle arrest in the G2/M phase, and apoptosis. Furthermore, alterations in the protein levels of G2/M cyclins such as CDK1, Cyclin E, and Cyclin B were also observed after treatment. Additionally, the administration of compounds c1 and c2 led to a noteworthy reduction in tumor size in nude mice.

**Conclusion:** In summary, the study demonstrated the anti-tumor effects of compounds c1 and c2 associated with the induction of oxidative stress and senescence-dependent mechanisms. These compounds can be further evaluated as potential anti-cancer agents for HCC.

**Figure:**


Control
Doxorubicin
c1
c2
MSH6
FEN1
KLF4
RPA3
ORC6
RFC5
MCM5
CDKN1B
RFC4
RFC3
CCNB1
SFN
CDC6
MCM4
CCNA2
E2F1
CCNE2
CDC25C
H3F3A
DUSP6
PTTG2
HIST1H3B
HIST1H3H
Liver Cancer
Tumor volume (mm3)
1500
1250
1000
750
500
250
0
0
10
20
30
Time(Days)
** p<0.05
**** p<0.00001
Control
c1
c2

# PO5-07
# Differential expression of MicroRNA-21 in MASLD and HCV-related hepatocellular carcinoma: a pilot study

Henrique Takematsu[1], Michele Gouvea[2], Isabel Veloso Alves Pereira[1], Jose Tadeu Stefano[1], João Renato Rebello Pinho[1], Claudia P. Oliveira[1]

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**Background and aims:** MicroRNA-21 (miR-21) stands out as one of the consistently elevated microRNAs in chronic liver diseases, including metabolic-associated steatotic liver disease (MASLD) and hepatocellular carcinoma (HCC), making it a promising diagnostic marker. MiR-21 is recognized as an oncomir, influencing cell proliferation and invasion while concurrently suppressing cellular apoptosis. Limited attention has been given to understanding the molecular drivers of hepatocarcinogenesis in the MASLD context compared to other HCC etiologies, such as hepatitis C virus (HCV) infection. As previously described in the literature, the ACVR2A mutation is identified as one of the hallmarks of MASLD-related HCC, and ACVR2A serves as a binding site for miR-21. This study aimed to compare miRNA 21, 29, 33a, 122-5p, 155, and 181a expression in the serum of MASLD-related HCC and HCV-related HCC.

**Method:** We evaluated 18 patients, divided into two groups: MASLD-related HCC (n = 11) and HCV-related HCC (n = 7). Anthropometric, biochemical, and clinical data were collected retrospectively, and serum samples were used for miRNA expression as follows: serum samples were stored at −80 °C and used for total RNA isolation. Total RNA extraction was conducted using TRIzol® LS reagent (Ambion® by Life Technologies, Carlsbad, CA, USA). Following quality analysis and quantification, a reverse transcription reaction was performed to synthesize complementary DNA (cDNA), which was subsequently employed in miRNA expression assays.

**Results:** Patients were predominantly male, with an average age of 64 years in MASLD-related HCC and 62 years in HCV-related HCC. ALT, AST, GGT, albumin, ALP, creatinine, bilirubin, and glucose levels were the same in both groups. All MASLD-related HCC patients were diabetic and had metabolic syndrome and systemic hypertension. AFP level was normal in 81% (n = 9) of MASLD-related HCC. miRNA 21 was higher in the serum of MASLD-related HCC compared with HCV-related HCC (p = 0, 05). miRNA 29, 33a, 122-5p, 155, and 181a expression did not differ between etiologies.

**Conclusion:** This is a pilot study that demonstrated higher expression of miR-21 in MASLD-related HCC when compared to HCV-related HCC. Despite the study's limitations, we can observe that miR-21 may exhibit differences in its expression, which should be taken into account when considering it as a tumor marker. Furthermore, the data align with existing literature, suggesting that miR-21 is a superior disease marker compared to AFP.

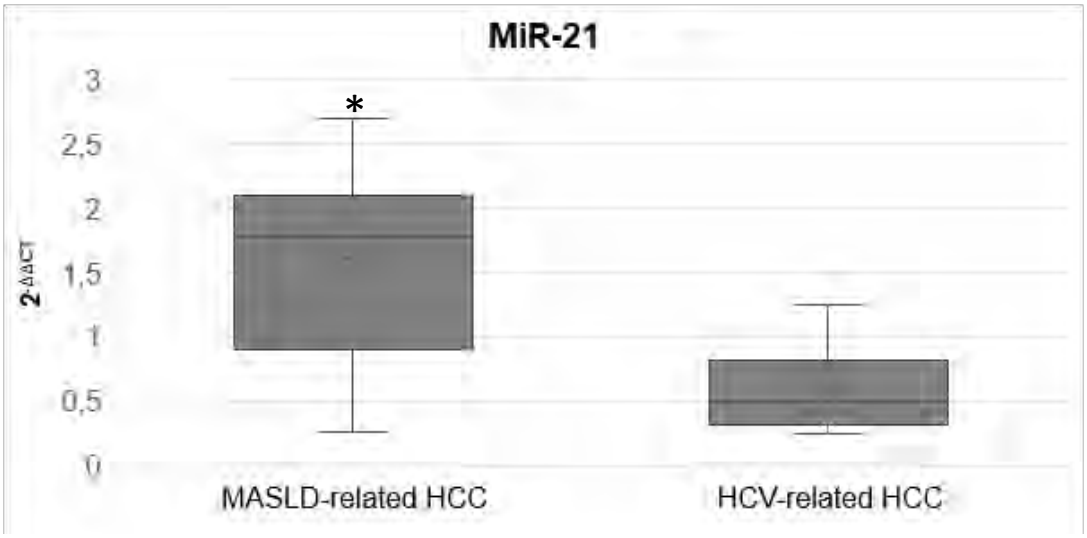


**Figure:**
* p = 0, 05‘

## PO5-11-YI

# Imparing proliferation, survival and invasiveness of intrahepatic cholangiocarcinoma through a novel natural hedgehog pathway inhibitor

Savino Paradiso[1], Guido Carpino[1], Deborah Quaglio[2], Francesca Ghirga[2], Chiara Di Meo[2], Luca Paoletti[2], Teresa De Luca[3], Matteo Franchitto[4], Lucia Di Marcotullio[5], Paola Infante[5], Eugenio Gaudio[1], Domenico Alvaro[3], Vincenzo Cardinale[3]

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**Background and aims:** Intrahepatic cholangiocarcinoma (**iCCA**) represents a rare and aggressive cancer that originate from the biliary tree bearing a fatal prognosis (5-years relative survival rate). Nowadays, it is a worldwide health issue that need an imperative resolution. Depending on its molecular, histological, and clinical various features, its subcategorization into small and *large bile duct iCCA* has been approved by World Health Organization (*ICD-O-3.2*). From a molecular perspective, there are several signaling pathways entailed in the genesis, promotion and aggressiveness, and diagnosis and prognosis of iCCA. Among them, **Hedgehog** (Hh) pathway plays a decisive function in tumor proliferation, survival and epithelial-mesenchymal transition. The main purpose of this study is to shed a light on a new Hh inhibitor, a natural product named Glabrescione B (GlaB), *in vitro* in iCCA established and primary cell lines.

**Method:** Trypan Blue Exclusion test have been used to assess the dose-response of free GlaB and hyaluronic acid (HA)-encapsulated GlaB, an inhibitor of Gli1 (a transcription factor and the final effector of Hh pathway). Western blot has been used to evaluate the target protein levels. Wound healing assay has been established to evaluate the migratory activity. Flow cytometry analyses has been used to assess cell death after treatments. All experiments have been conducted in n.3 experimental replicates.

**Results:** Our research reports a mitigation in iCCA cell rate proliferation, migration, and decrease of Gli1 levels in a dose- and time-dependent manner after both free GlaB and HA-GlaB (for better deliver the drug in the site of injury) treatments ($p < 0.05$), leading to a significant reduction of invasiveness and aggressiveness in cancer cells. Further, flow cytometry preliminary data indicates an induction of cell death after drug administration compared to controls.

**Conclusion:** These data provide the basis for additional *in vitro* investigations and *in vivo* pre-clinical studies for the management of iCCA.

**Figure:**

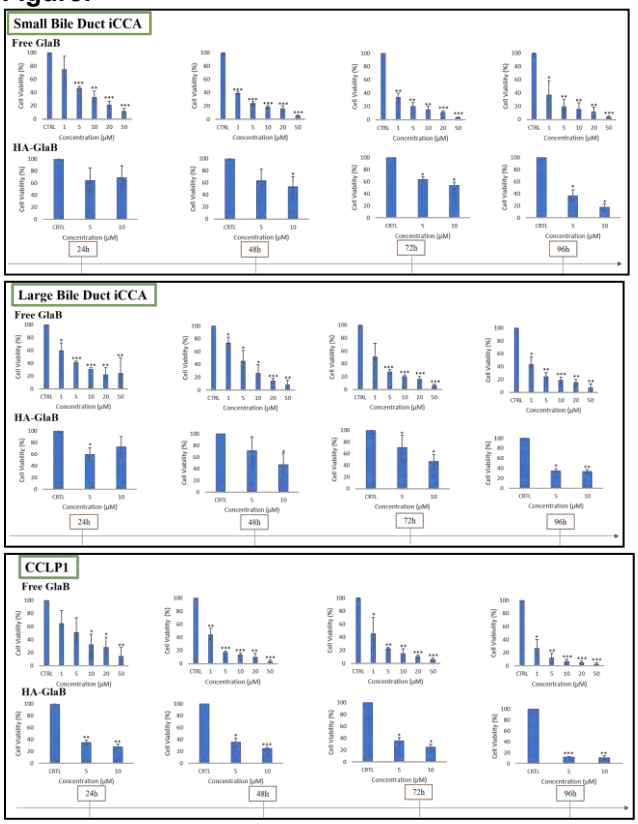


Fig.1 *Proliferation rate of iCCA cell lines subjected to free GlaB or HA-GlaB treatment.*

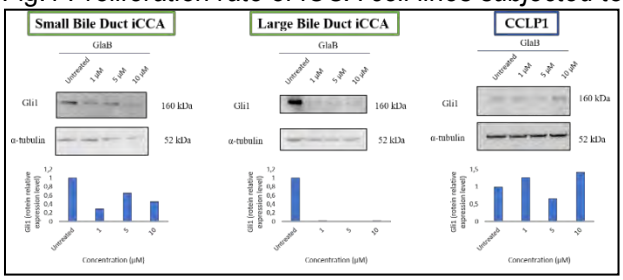


Fig.2 *Western blot and quantification by densitometry of iCCA cell lines after free GlaB treatment.*

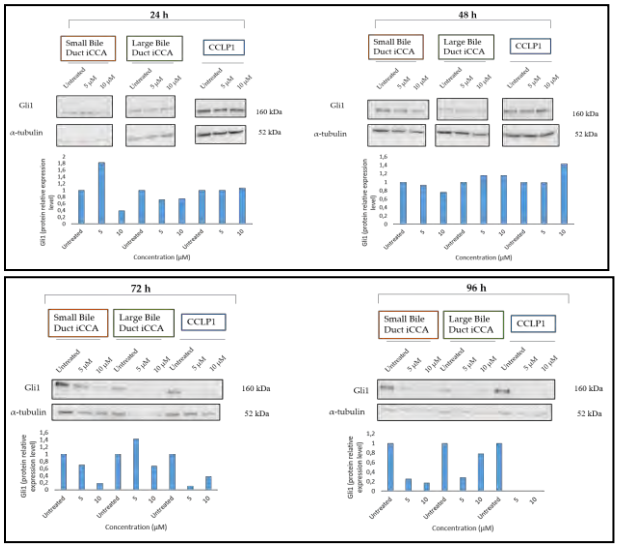


*Fig.3 Western blot and quantification by densitometry of iCCA cell lines after HA-GlaB treatment.*

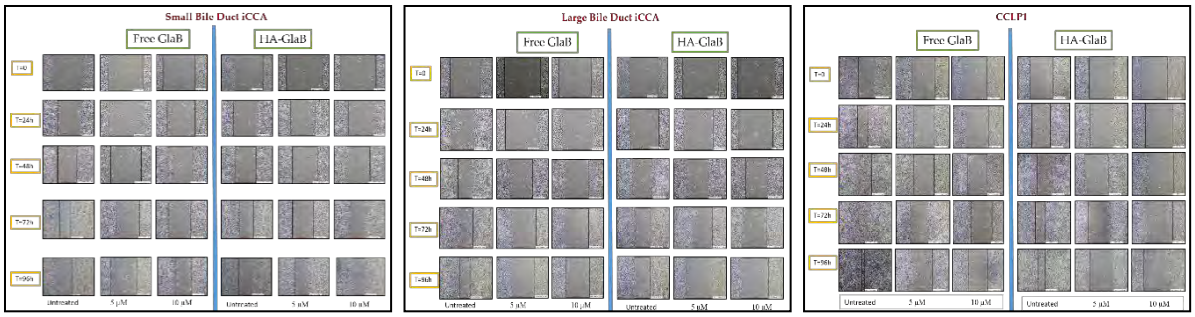


*Fig.4 Representative area for wound-healing assay of iCCA cell lines after different time points of scratch, treated with Free GlaB and HA-GlaB. Scale bar, 200 µm.*

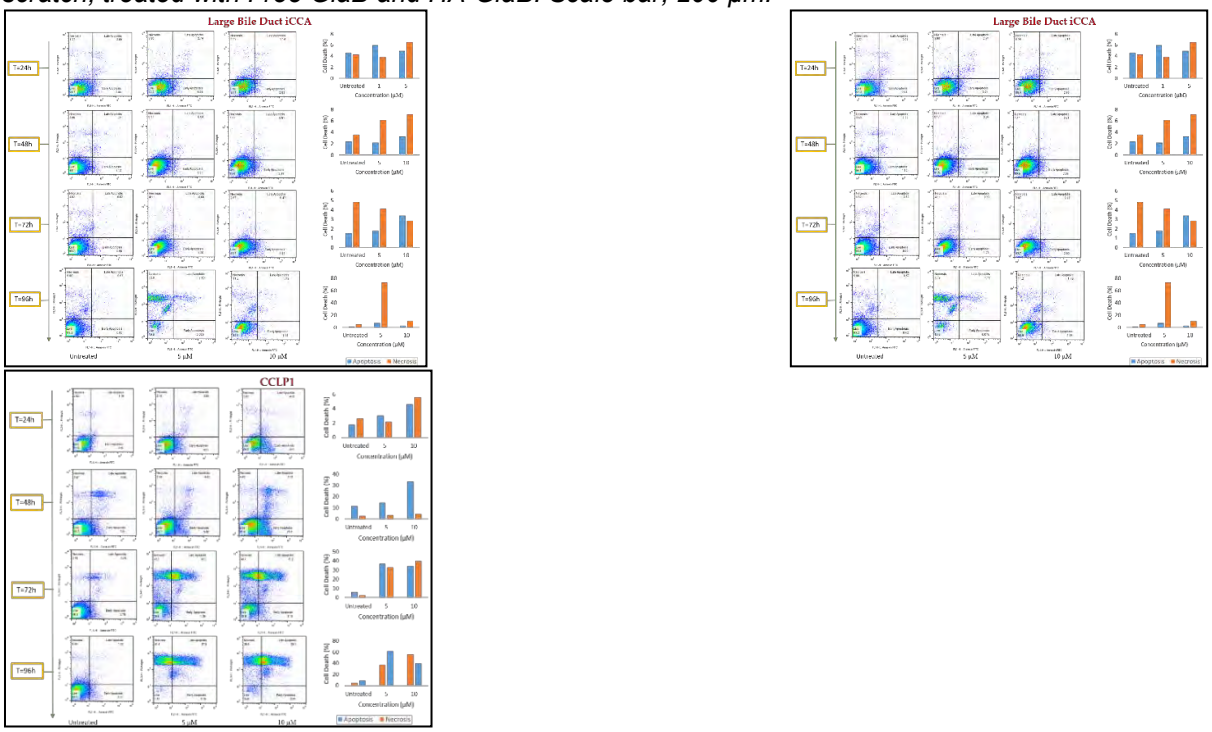


*Fig.5 Cell death (%) in all cell lines after different time points of treatment by flow cytometry analyses.*

# PO5-12-YI
# Role of ganglioside GD2 in the stem-like compartment of intrahepatic cholangiocarcinoma

Antonella Mannini[1], Mirella Pastore[1], Margherita Correnti[2], Tiziano Lottini[1], Benedetta Piombanti[1], Ignazia Tusa[1], Elisabetta Rovida[1], Cedric Coulouarn[3], Jesper Andersen[4], Monika Lewinska[4], V Lokesh Battula[5], Yuan Bin[5], Massimo Aureli[2], Emma V Carsana[2], Claudia Campani[1], Caterina Peraldo Neia[6], Paola Ostano[6], Luca Di Tommaso[7], Fabio Marra[3], Chiara Raggi[1]

*[1]University of Florence*, *[2]University of Milan*, *[3]University of Rennes*, *[4]Copenhagen University, København, Denmark*, *[5]MD Anderson Cancer Center*, *[6]Fondazione Tempia*, *[7]Humanitas Insitute*

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**Background and aims:** Among the ganglioside family (sialic acid-containing glycosphingolipids), GD2 has been recently investigated as a potential cancer stem cell (CSC) biomarker in several tumor types. However, the possible role of GD2 and its biosynthetic enzyme, GD3 synthase (GD3S), in intrahepatic the cholangiocarcinoma (iCCA) stem subset is currently unknown.

**Method:** Stem-like subset of iCCA cell lines (HUCCT1, CCLP1) was enriched by sphere culture (SPH) and compared to monolayer parental cells (MON). iCCA ganglioside (GS) profiles were evaluated by chromatographic analytical procedures, after feeding with radioactive sphingosine. Plasma membrane GD2 expression was evaluated by FACS, while GS biosynthesis enzymes were analyzed by RT-qPCR during spherogenesis. The modulation of stem features by GS was investigated *in vitro* and *in vivo* by using iCCA GD3S-transfected cells, and corroborated by global transcriptomic analysis.

**Results:** In both iCCA lines, SPH showed severe changes in GS composition, including an increased content of GM3 and GD1a, reduction of GM2, and, among complex GS, increase or appearance of GD2. These findings were corroborated by high levels of GM3 synthase as well as GD3- and GM2/GD2 synthase expression. Cancer-stem features related to GD2 availability depend on the GD3S, the synthase that provides the precursor (GD3) to produce GD2. Significant correlations between GD3S expression and lymph node invasion were found in a published iCCA cohort by exploring the possible relevance of GD3S involvement in the clinical setting. After GD3-stable transfection, iCCA cells overexpressing GD3S showed *in vitro* enhanced sphere-forming ability, invasive properties as well as higher drug resistance compared to the transfected control. *In vivo* experiment, CCLP1 cells overexpressing GD3S developed a tumor mass volume 2-3 fold greater compared to the control. By global transcriptomic analysis, ontology investigations identified 74 processes shared by the iCCA-SPH and GD3S-transfected cells, with enrichment for development and morphogenesis processes, signaling, in particular MAPK pathway and locomotion.

**Conclusion:** We demonstrate that the iCCA stem-like properties are related to the GS synthetic pathways and patterns. GD3 synthase and GD2 ganglioside could represent potential markers for iCCA stem phenotype.

## PO5-13
# Aptamers targeting HuR SUMOylation as a potential therapy for liver cancer

Patricia Peña-San Felix[1], Blanca Baños Jaime[2], Laura Corrales-Guerrero[2], Elena Martín Palma[3], Sofia Lachiondo-Ortega[1], Leidy Estefanía Zapata-Pavas[1], Claudia M. Rejano Gordillo[1], Irene Díaz-Moreno[2], Víctor. M González[3], María Luz Martínez-Chantar[1 4]

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**Background and aims:** Liver cancer ranks as the sixth most prevalent cancer worldwide and the second cause of cancer-related deaths. Nowadays, surgery and liver transplant persist as main treatment options. However, a considerable percentage of liver cancer patients cannot opt for these treatments due to their characteristics. Although new therapies have emerged in recent years, some still in clinical phases, they predominantly act systemically, emphasizing the need for targeted and specific therapeutic approaches. In this study, we propose the development of aptamers to specifically block HuR SUMOylation in the liver. Aptamers are DNA or RNA molecules that acquire a three-dimensional conformation, giving them affinity towards a specific target-in this case, the SUMOylation sites of HuR. HuR is an RNA-binding protein involved in various processes contributing to the acquisition of a tumorigenic character, including cell proliferation, angiogenesis, and invasion. Our previous findings indicated increased HuR SUMOylation in tumours from human patients with liver cancer compared to surrounding tissue, as well as in human cell lines and murine models of the disease. This HuR SUMOylation contributes to proliferation and invasion, while its absence induces a senescent phenotype, characterized by mitochondrial and endoplasmic reticulum dysfunction.

**Method:** Within our group, we have identified the main sites of HuR SUMOylation. Specific aptamers against these sites have been selected using the SELEX (Systematic Evolution of Ligands by EXponential Enrichment) method, enabling determination of aptamers with the highest affinity and specificity for a specific target. Initially, these aptamers were computationally analysed to define their stability and interaction efficiency with HuR. To evaluate the efficiency of the aptamers in vitro, we selected the human hepatoma cell line Huh7, which exhibits high levels of SUMOylated HuR. We compared the impact of these aptamers on proliferation in Huh7 cells constitutively expressing the wild-type version of HuR (Huh7 HuR WT) versus those expressing a version with mutated SUMOylation sites (Huh7 HuR Mut).
**Results:** Aptamer treatment resulted in a reduction in cell proliferation in Huh7 HuR WT cells, mirroring the phenotype observed in the Huh7 HuR Mut cells, where the aptamers showed no effect. Moreover, aptamer treatment increased sensitivity to palbociclib. Concerning the mechanistic aspects, the upregulation of oxidative stress markers suggests an induction of mitochondrial dysfunction in response to aptamer treatment.

**Conclusion:** All these findings highlight the potential of aptamers targeting HuR SUMOylation as a novel targeted and specific therapeutic approach for liver cancer.

## PO5-17
# Tumor-derived extracellular vesicles: ultrasensitive detection using mid-infrared resonant nanostructures

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**Background and aims:** Liver cancer is a major cause of cancer worldwide. Among them, hepatocellular carcinoma (HCC) is currently the seventh most common type of solid neoplasm and the second most frequent cause of cancer-related deaths. This tumor frequently develops in the setting of cirrhosis and chronic liver diseases. The incidence rates have been changing profoundly over the past decades. The reasons for these changes lie in the hepatitis B virus vaccination programme, the availability of nucleos (t)ide analogues for HBV, and direct-acting antiviral drugs for hepatitis C virus. On the other hand, metabolic syndrome, obesity, Type II diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) are increasing and represent one of the major risk factors for the development of HCC. In this scenario, the role of surveillance of patients with cirrhosis or chronic liver diseases is clear, with the aim of early diagnosis of HCC and the possibility of effective treatment. The recommended investigation is ultrasonography with serum alfa-fetoprotein (AFP) levels. Unfortunately, this biomarker demonstrates limited performance: AFP is only 80% specific in diagnosing HCC and can be normal in about 40% of patients with early disease, leading to an increasing demand for novel circulating markers specific to HCC. In recent years, extensive literature has been produced on new possible biomarkers for HCC. The aim of our study is instead aimed to evaluating a new device.

**Method:** We used Fourier Transform Infrared (FTIR) spectroscopy, in Attenuated Total Reflection (ATR) mode, to analyze plasma, red blood cells (RBCs), and extracellular vesicles (EVs) from 14 cirrhotic patients (control) and 10 HCC patients with cirrhosis.

**Results:** In the Amide II region of the EVs' spectra, wich behaved similarly to AFP in identifying patients (AUC = 0.75), we found substantial differences. The combination of these two markers performs better than AFP alone with an astounding AUC of 0.89 (95% CI 0.75-1.0), wich is more intriguing. Our group created a metasurface with double-resonant nanoantennas (NAs) for Amide I and II targeting and simultaneous mass sensing to overcome EV sample purity issue and select cancer-related EVs. Epcam-specific antibody-conjugated NAs were used to purify and trap EVs, demonstrating differences in Epcam-presenting vescicles between HCC patients and controls.

**Conclusion:** Our study highlights the potential of FTIR spectroscopy and plasmonic nanostructures in HCC diagnosis, offering a promising alternative to existing circulating markers.

## PO5-18-YI

# Decoding human intrahepatic cholangiocarcinoma metabolism: unveiling the impact of SLC2A3 on aggressiveness and prognosis

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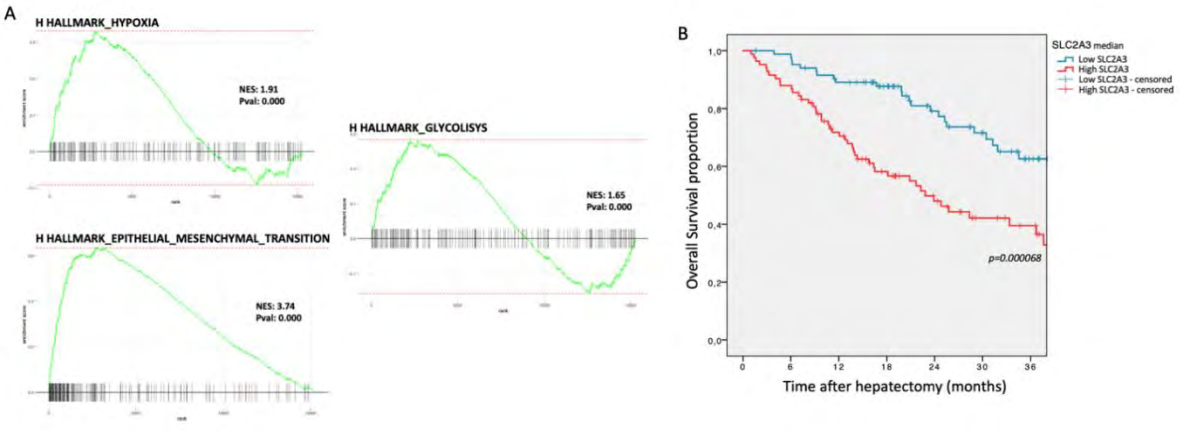
**Background and aims:** Intrahepatic Cholangiocarcinoma (iCCA) is a cancer of the biliary epithelium with a poor prognosis and limited therapies. Understanding iCCA pathophysiology is crucial for developing effective treatments. In this scenario, tumor metabolism reprogramming, a cancer hallmark, is of interest due to its impact on tumor aggressiveness and therapy resistance. Herein, we investigated the role of upregulated glycolysis in iCCA and its interplay with aggressiveness and prognosis.

**Method:** Normal cholangiocytes (n = 5) and iCCA cells (n = 25) were isolated from patients resected at the Division of Hepatobiliary and General Surgery, Humanitas Clinical Institute. iCCA cell supernatants were analyzed by mass spectrometry-based targeted and untargeted metabolomic approaches. RNAseq and RT-PCR analyses were carried out to identify altered metabolic pathways in iCCA. An iCCA cell line (HuH28) was cultured under hypoxic conditions and migration assay was performed. RNAseq and clinical data of iCCA patients were obtained from a public dataset (OEP001105).

**Results:** The metabolomic analysis revealed that iCCA primary cells displayed elevated mitochondrial activity compared to normal cholangiocytes, with increased glutamine and glucose uptake. Moreover, RNAseq analysis unveiled changes in the glycolytic pathway, along with upregulation in hypoxia and epithelial-to-mesenchymal transition (EMT) pathways in iCCA cells (**Fig.1A**).To better elucidate the role of glycolysis, we analyzed the expression by RT-PCR of two main glucose transporters, observing a down-regulation of SLC2A1 and a significant upregulation of SLC2A3 in iCCA cells, compared to cholangiocytes. Moreover, glucose uptake assays showed a positive correlation between glucose intake and SLC2A3 expression, underscoring its role in mediating glucose metabolism in iCCA cells, than SLC2A1.To gain insights into the interplay between hypoxia and glycolysis, HuH28 cells were cultured under hypoxic conditions, showing increased migratory ability and upregulation of SLC2A3 and NCAD expression, compared to normoxia. We further explored the link between glycolysis and EMT, demonstrating a correlation between SLC2A3 expression and EMT markers, as ZEB1 and NCAD, in iCCA. To elucidate the impact of SLC2A3 on iCCA prognosis, patients (n = 151) from public RNAseq dataset were divided into low- and high-SLC2A3 expressions based on the median value. Notably, high-SLC2A3 patients displayed significantly poorer survival outcomes than low-expressing SLC2A3 patients (**Fig.1B**).

**Conclusion:** This study revealed the significant upregulation of SLC2A3 in iCCA and its association with hypoxia and EMT, unveiling the interplay between metabolic reprogramming and tumor aggressiveness in iCCA. These findings emphasize the therapeutic potential of targeting SLC2A3, offering a promising avenue for intervention in iCCA.

**Figure:**


A
H HALLMARK_HYPOXIA
NES: 1.91
Pval: 0.000
H HALLMARK_GLYCOLISYS
NES: 1.65
Pval: 0.000
H HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
NES: 3.74
Pval: 0.000
B
SLC2A3 median
Low SLC2A3
High SLC2A3
Low SLC2A3 - censored
High SLC2A3 - censored
Overall Survival proportion
p=0.000068
Time after hepatectomy (months)

# PO6-01
# Characterization of Entosis, a cell cannibalism process, in hepatocellular carcinoma

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**Background and aims:** Entosis is a process that leads to the formation of cell-in-cell structures commonly observed in a wide range of human cancers and mainly associated with poor prognosis. Here, we aim at characterizing entosis in hepatocellular carcinoma (HCC), deciphering molecular pathways involved and addressing its link with prognosis in patients.

**Method:** Cultured cells (Hep3B, Huh7, Huh6 or HepG2) were subjected to stress conditions known to favor entosis in breast cancer cells such as nutrient deprivation or matrix detachment. MCF-7 human breast cancer cells were used as a positive control. Cell internalization was analyzed by confocal microscopy after immunostaining of nuclei, F-actin and β-catenin to visualize nucleus deformation, cell periphery and shape respectively. Characterization of entosis stages was analyzed using live and correlative light-scanning electron microscopies. Molecular pathways involved in entosis formation were addressed using proteomic profiling of entotic cells and RNA interference *in vitro* in cultured HCC cells and *in vivo* in mouse xenografts. Entosis was assessed in HCC patient samples using IHC.

**Results:** Our results demonstrate that liver cancer cells are prone to perform entosis upon proper stimuli such as nutrient deprivation or matrix detachment. We identified the loss of Rnd3/RhoE as an efficient inducer of this mechanism, both in vitro and in vivo. Using time-lapse microscopy, we found that entosis initiated by the contact between two cells, and then the internalization was marked by a high concentration of F-actin at the border between the two cells. The nucleus of the outer cell acquired a change in its shape due to the nucleus of the inner cell that pushes and deforms it. The final stage was characterized by the degradation of the inner cell. Using correlative light-scanning electron microscopy, we found that the internalized cell has a rounded shape and appeared to be denser than the outer cell, with apparent cytoskeletal filaments. Using RNAi-based knock-down, we demonstrated that this process depends on RhoA/ROCK pathway, but not on E-cadherin. The proteomic profiling of entotic cells allowed us to identify LAMP1 as a protein upregulated by Rnd3/RhoE silencing and implicated not only in the degradation final stage of entosis, but also in the full mechanism. Moreover, we found a positive correlation between the presence of entotic cells and the metastatic potential of tumors in human patient samples.

**Conclusion:** In this study, we highlighted that liver cancer cells are prone to perform entosis upon proper stimuli. We revealed that Rnd3/RhoE loss favors entosis in HCC tumors. Altogether, our data suggest the involvement of entosis in HCC progression and highlight a new perspective for entosis analysis in medicine research as a novel therapeutic target.

## PO6-02
## Involvment of the potassium channel ERG1 in cholangiocarcinoma

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**Background and aims:** Due to the lack of proper biomarkers and potentially effective treatments, the management of cholangiocarcinoma (CCA) is still challenging. Ion channels have been proven to be novel biomarkers and new targets for cancer therapy, due to their easy druggability. The voltage-gated K+ channel hERG1 exerts pleiotropic effects in cancer cells. This study explored the role of hERG1 in the biology of intrahepatic CCA (iCCA).

**Method:** Validation of hERG1 in iCCA tissues was performed in TCGA database. In vitro experiments were conducted to estimate the impact of hERG1 inhibition on cell function in iCCA cell lines (HUCCT1, CCLP1, CCA4).

**Results:** A significant difference in hERG1 gene expression was observed between iCCA and normal tissue samples. Similarly, iCCA cell lines showed significantly higher protein content of hERG1 compared to normal cholangiocytes (NHC3). Treatment with E4031, a selective hERG1 inhibitor, showed a limited impact on cell growth, but a substantial reduction of the invasive capabilities of iCCA cells. Immunoprecipitation assays and immunofluorescence revealed the formation of an active macromolecular complex with β1 integrin responsible for VEGF-A activation through AKT signaling. Treatment with a bispecific antibody (scDb: single-chain Diabody) that binds the hERG1-β1 complex, negatively impacted the invasiveness of iCCA cells as well as expression of genes regulating epithelial to mesenchymal transition. In vitro co-treatment with scDb and cisplatin-gemcitabine, significantly reduced growth of iCCA cells.

**Conclusion:** This study indicates that hERG1 may be relevant in promoting the malignant characteristics of iCCA.

# PO6-08-YI
# Cancer associated fibroblast plasticity and interaction with the tumour microenvironment in cholangiocarcinoma

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**Background and aims:** Cholangiocarcinoma (CCA) is a lethal malignancy of the liver and bile ducts. A main characteristic of CCA is the presence of a complex and dynamic tumor microenvironment (TME), composed primarily of cancer associated fibroblasts (CAFs). CAFs are actively involved in extracellular matrix remodeling, neo-angiogenesis, and metastasis-mechanisms that contribute to cholangiocarcinogenesis. As such, transcriptomically unique CAF subpopulations (namely myofibroblastic CAFs, inflammatory CAFs and antigen presenting CAFs) have been identified to co-exist in the CCA TME. Furthermore, it has been hypothesized that highly plastic CAFs possess the ability alter their phenotype in order to support tumorigenesis.

**Method:** CAFs were isolated from patients presenting with CCA (n = 10). Bulk RNA-sequencing was performed to transcriptomically characterize primary CAFs. Supernatant of isolated primary CAFs was applied to human umbilical vein endothelial cells (HUVECs) that were then evaluated for tube-formation and/or proliferation. To investigate how CAF plasticity alters response in the TME, CAF lines were immortalized (imCAFs) with SV40 Large T antigen (n = 5) evaluated for changes in transcriptomic, secretory profiles and their ability to interact with tumor cells and endothelial cells upon inhibition of the JAK/STAT pathway.

**Results:** CAFs isolated from patient samples revealed unique transcriptomic profiles. Exposing HUVECs to CAF supernatants indicated that CAFs are able to successfully sustain HUVEC proliferation and induce the formation of unique networks in a tube formation assay. Furthermore, selected CAF lines were successfully immortalized and retained transcriptomic features of their parental lines and the ability to interact with endothelial cells. Analysis of supernatants revealed that imCAFs upregulate secretion of VEGF-C but downregulate expression of other angiogenic factors including insulin growth factor binding protein 2 (IGFBP-2), angiogenin and platelet derived growth factor (PDGF-AA). Lastly, plasticity mechanisms were investigated by inhibition of the JAK/STAT pathway in imCAFs which revealed a significant increase in myofibroblastic ACTA2 expression decreased ability to induce endothelial cell proliferation.

**Conclusion:** Heterogenous CAF subpopulations that co-exist in the TME are highly plastic cells and possess the ability to interact with many cell types. Here we focus on a rather unexplored interaction between CAFs and endothelial cells in the TME. Although widely characterized as chemotherapeutically resistant and bearing a hypoxic microenvironment due to the presence of an exacerbated stroma, CAFs possess the ability to interact with tumour endothelial cells. Manipulating the transcriptomic profile of CAFs to increase this interaction opens up new perspectives to increase therapeutic efficacy.

## PO6-09-YI
# Unveiling Tigar SUMOylation in the metabolic response of liver cancer

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*[1]CIC bioGUNE-Centro de Investigación Cooperativa en Biociencias, Derio, Spain*, *[2]University of Campania Studies "Luigi Vanvitelli", Napoli, Italy*, *[3]University of Padua, Padova, Italy*

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**Background and aims:** Tumors, including hepatocellular carcinoma (HCC), undergo profound changes in metabolic pathways to fuel their growth and survival. In this study, we introduce a revolucionary non-tumor-suppressive role of p53: the metabolic reprogramming of HCC through one of its target genes, TP53 Induced Glycolysis Regulatory Phosphatase (TIGAR). While it is well-established that TIGAR's protumorigenic role involves diverting glucose from energy production towards nucleotide synthesis and DNA lesion repair in cancer cells, the mechanisms behind its activation remain elusive. Recently, SUMOylation has emerged as a pivotal post-translational modification (PTM) in liver cancer progression.

**Method:** In this study a comprehensive SUMO interactome analysis was conducted via GST-tagged SUMO Binding Entities (SUBEs) protein pulldown in several human hepatoma cell lines (HuH7, PLC, HepG2, and SNU449).

**Results:** Among the identified proteins, TIGAR exhibited a notably higher proportion of sumoylation, particularly in the SNU449 cell line, which subsequently became the primary focus of our study. Subsequently, employing diverse bioinformatic tools, we identified potential sites on the TIGAR protein susceptible to sumoylation and the development of stable cell lines expressing both wildtype and mutant versions of TIGAR. We successfully demonstrated a reduction in the level of sumoylated TIGAR upon introduction of the mutation. In addition, the cell line expressing mutant TIGAR showed less proliferation, migration, invasion and sferoids formation. We found out that the absent of TIGAR sumoylation affected the pentose phosphate shunt with an increased NADP/NADPH ratio and reduced GSH/GSSG ratio, which revealed more oxidative stress in this cells.

**Conclusion:** In conclusion, SUMOylation constitutes a novel mechanism of TIGAR regulation that could be potentially exploited as a therapeutic strategy for liver cancer, thus highlighting the importance of PTMs as disease targets. Considering that both TIGAR and SUMOylation may also play beneficial roles for correct cellular function, we strongly believe that inhibiting TIGAR SUMOylation, alone or in combination with currently approved therapies, will lead to the regression of hepatic tumors.

# PO6-10
# Circular RNAs in hepatocellular carcinoma: the study of hsa_circ_0062682

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*[1]University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, CFGBC, Ljubljana, Slovenia*, *[2]University Medical Centre Ljubljana, Department of Gastroenterology, Ljubljana, Slovenia*, *[3]University Medical Centre Ljubljana, Department of Abdominal Surgery, Division of Hepatobiliary Surgery and Liver Transplantation, Ljubljana, Slovenia*, *[4]University Medical Center Maribor, Department of Abdominal and General Surgery, Ljubljana, Slovenia*, *[5]Jožef Stefan Institute, Department of Biochemistry and Molecular and Structural Biology, Ljubljana, Slovenia*, *[6]BIA Separations CRO, Labena d.o.o, Ljubljana, Slovenia*, *[7]University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Ljubljana, Slovenia*

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**Background and aims:** Several circular RNAs (circRNAs) have been shown to be differentially expressed in hepatocellular carcinoma (HCC) and have a role in the HCC pathology. CircRNAs also represent promising biomarkers due to their presence in liquid biopsies and stability. Herein, we report the characterization of a circRNA hsa_circ_0062682 in HCC and evaluate its diagnostic potential in Slovenian HCC patients.

**Method:** We compared the expression of selected circRNAs in plasma, liver tumor and paratumor samples in published transcriptome datasets with the Slovenian HCC. We evaluated the oncogenic potential of the circRNA hsa_circ_0062682 in cell lines using transcriptome analysis and various functional cell-based assays, such as proliferation, migration, invasion, and colony formation assays. We identified potential binding partners using biotinylated oligonucleotide pulldown coupled with mass spectrometry and RNA immunoprecipitation.

**Results:** By analyzing microarray datasets in GEO, we identified hsa_circ_0062682 as upregulated in HCC tumor tissue. Employing various functional assays, we both increased and suppressed the expression of hsa_circ_0062682 in diverse HCC cell lines, confirming its oncogenic potential. A cell-type-specific response to modulated expression of hsa_circ_0062682 was observed in sorafenib sensitivity, migratory ability, and differential localization of selected proteins. The exploration of the transcriptome by integrating pathway enrichment analysis and gene set enrichment analysis unveiled systemic alterations triggered by perturbations in hsa_circ_0062682 expressions. Incorporating a proteomics approach, we unveiled the protein binding partners of hsa_circ_0062682, and subsequent RNA immunoprecipitation affirmed its interaction with YBX1, a known oncogene. Intriguingly, the expression of this circRNA was downregulated in tumors and plasma of the Slovenian HCC cohort, characterized by a metabolic and alcohol-associated etiology. We posit that this disparity may stem from the diverse etiologies and molecular subtypes present in the HCC cohorts utilized in microarray datasets. Furthermore, we assessed the expression of other circRNAs in our cohort. We are developing the protocol for circRNA expression analysis using long-read sequencing.

**Conclusion:** Our data suggest that hsa_circ_0062682 promotes oncogenesis in HCC. The discrepancy in circRNA expression in HCC tumors between cohorts could be due to different etiologies of HCC patients.

# PO6-11-YI
# Caspases compromise SLU7 and UPF1 stability and nonsense-mediated RNA decay activity during hepatocarcinogenesis

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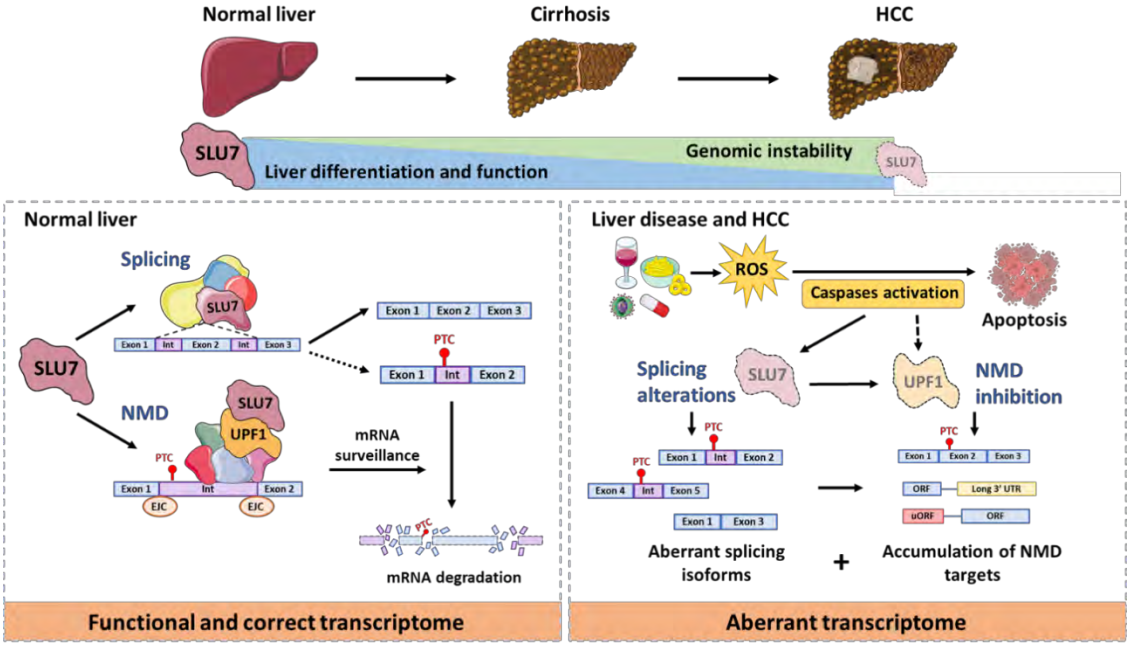
**Background and aims:** Cellular transcriptome homeostasis depends on transcription and splicing. Moreover, the fidelity of gene expression, essential to preserve cellular identity and function is secured by different quality control mechanisms including nonsense-mediated RNA decay (NMD). Our previous findings demonstrated that the expression of the splicing factor SLU7 is reduced in hepatocellular carcinoma (HCC) and the damaged liver, contributing to hepatocellular de-differentiation and genome instability, associated to transcription and splicing changes. Here demonstrate that SLU7 plays a role in NMD which is inhibited in the damaged liver and in HCC. Importantly, we also decipher the mechanism by which SLU7 is downregulated during the progression of liver disease.

**Method:** We used several human extra-hepatic cancer and HCC cell lines, as well as mouse primary hepatocytes, SLU7 specific siRNAs, apoptosis activators, the antioxidant N-acetylcysteine and the pan-caspase inhibitor zVAD-fmk. Animal models: administration of Fas agonistic antibody Jo2 or acetaminophen (APAP), and Mdr2-/- mice. RNA-seq analysis at the transcript level by Kallisto and SLEUTH, qPCR, Western blot, and immunoprecipitation/mass spectrometry assays. Chemiluminescence-based NMD reporter assay to measure NMD activity. LIHC-TCGA RNA-seq data. Statistical analyses with GraphPad Prism 8.0.1.

**Results:** TCGA database analysis demonstrate the accumulation in HCC of NMD-target transcripts suggesting the inhibition of NMD activity. RNA-seq analysis in PLC/PRF/5 cells, as well as PCR and qPCR validation in different cancer cell lines revealed that SLU7 depletion induces a significant accumulation of NMD-targets and intron retention isoforms, in parallel to the upregulation of NMD factors, all events associated with NMD inhibition. Moreover, we demonstrate that SLU7 interacts with and stabilizes the NMD core effector UPF1, and that SLU7 is required for a correct NMD activity. Importantly, we found that in two animal models of acute liver injury (Jo2 and APAP) and during the process of hepatocarcinogenesis in Mdr2-/- mice, NMD is inhibited, resulting in the accumulation of different NMD targets, in parallel to the downregulation of UPF1 and SLU7 proteins.
Remarkably, we provide in vitro and in vivo evidence showing that caspases activation would be responsible of the cleavage and degradation of SLU7 observed during the process of hepatocarcinogenesis in animal models and patients.

**Conclusion:** Here we identify the downregulation of UPF1 and the inhibition of NMD as a new molecular pathway contributing to the malignant reshaping of liver transcriptome. Moreover, and importantly, we uncover caspases activation as the mechanism responsible for the downregulation of SLU7 expression during liver disease progression, representing a new link between apoptosis and hepatocarcinogenesis.

**Figure:**


Normal liver
Cirrhosis
HCC
SLU7
Liver differentiation and function
Genomic instability
SLU7
Normal liver
Splicing
SLU7
Exon 1 Int Exon 2 Int Exon 3
SLU7
NMD
SLU7
UPF1
PTC
Exon 1 Int Exon 2
EJC
EJC
Exon 1 Exon 2 Exon 3
PTC
Exon 1 Int Exon 2
mRNA surveillance
PTC
mRNA degradation
Functional and correct transcriptome
Liver disease and HCC
ROS
Caspases activation
Apoptosis
Splicing alterations
SLU7
UPF1
NMD inhibition
PTC
Exon 1 Int Exon 2
PTC
Exon 4 Int Exon 5
Exon 1 Exon 3
Aberrant splicing isoforms
+
PTC
Exon 1 Exon 2 Exon 3
ORF
Long 3' UTR
uORF
ORF
Accumulation of NMD targets
Aberrant transcriptome

## PO6-15
# Impact of hepatitis C virus on reovirus immunotherapy for hepatocellular carcinoma

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**Background and aims:** Chronic hepatitis C virus (HCV) confers a major risk for hepatocellular carcinoma (HCC). Despite successful treatment of HCV with direct acting antivirals (DAAs), cohorts of HCC patients remain with undiagnosed infections or with advanced HCC who are deemed unlikely to benefit from viral clearance. Oncolytic viruses (OVs) are an attractive emerging therapeutic option for HCC, however chronic HCV infection can theoretically directly disrupt pathways for OV immunotherapy. In addition, following cure of HCV a signature of persistent inflammation can remain, rendering patients potentially refractory to further stimulation by immunotherapies.

**Method:** We explored the impact of HCV proteins on the efficacy of OV immunotherapy, using reovirus-conditioned media (RCM) as a model of reovirus-induced inflammation. The effect of subgenomic HCV models on HCC-specific CD8+ T cell cytotoxicity was investigated, with a focus on dendritic cell (DC) maturation and antigen processing.

**Results:** RCM augments CD8+ T cell cytotoxicity against HCC cells but to a lesser extent when HCV proteins are present. RCM rescues an HCV-induced deficit in immunoproteasome subunit expression. While the most abundant peptides in the MHC-I-presented immunopeptidome are similar between HCV-bearing and parental cells, RCM-induced surface MHC-I expression and abundance of some highly immunogenic peptides are reduced by HCV. Curing HCV did not fully restore HCC immunogenicity.

**Conclusion:** In conclusion, HCV may negatively impact the efficacy of OV immunotherapies by dampening OV-induced inflammation and immunostimulation, rendering HCV positive tumours less immunogenic and therefore less likely to be killed by OV immunotherapy. The personalised management of HCV infections prior to OV immunotherapy may improve HCC-HCV outcomes.

# PO6-17
# The nuclear receptor TR4 promotes progression toward late-stage metabolic dysfunction-associated steatotic liver disease

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**Background and aims:** Metabolic dysfunction-associated steatotic liver disease is a type of liver condition marked by fat accumulation in the liver due to factors associated with metabolic disorders. This progressive disease can lead to fibrosis and liver cancer in its advanced stages. The mechanisms driving these severe outcomes are not fully understood. Nuclear receptors, such as the testicular receptor 4 (TR4), function as ligand-activated transcription factors. TR4 is involved in several physiological functions, including metabolism. It has also been linked to the development of certain cancers highlighting its importance in cell growth and differentiation. Here, we explore the function of TR4 in the advancement of metabolic dysfunction-associated steatotic liver disease, focusing specifically on its involvement in the fibrotic stages and its possible role in the progression to liver cancer.

**Method:** Mice with either TR4 loss or gain of function were subjected to a Western diet for 26 weeks. The extent of disease progression was assessed by examining tissue morphology using Haematoxylin-eosin and Sirius red staining, along with gene expression analysis of TR4. Disease characterization was performed in mice with altered TR4 function including wild-type controls, treated with either a vehicle or a liver-specific carcinogen. These mice were then fed either a pro-fibrotic high-fat diet or standard chow for up to 24 weeks. Subsequently, liver histology was evaluated, and serum markers were analysed. Additionally, a qPCR was conducted for genes associated with fibrosis.

**Results:** Mice on the Western diet demonstrated pronounced hepatic steatosis in Haematoxylin-eosin-stained sections. TR4 gain of function mice displayed increased fibrosis, as revealed by Sirius red staining. Conversely, TR4 knockout mice showed a potential reduction in liver fibrosis. A qPCR analysis revealed that TR4 was upregulated in mice consuming a Western diet compared to those on standard chow diets, indicating TR4's involvement in the advancement of metabolic dysfunction-associated steatotic liver disease.
Liver histology in mice fed with the pro-fibrotic high-fat diet and treated with carcinogen, confirmed the presence of steatosis and extensive fibrosis. Measurements of liver triglycerides indicated a significant accumulation of lipids in mice on the high-fat diet versus the control group. The levels of liver damage and metabolic markers in the serum were similar across all treated mice, independent of their TR4 expression. Furthermore, qPCR in the livers of these mice corroborated the expression of genes specific to fibrosis.

**Conclusion:** This study emphasizes the significance of the nuclear receptor TR4 as a potential crucial contributor to metabolic dysfunction-associated steatotic liver disease, suggesting its potential as an attractive target for drug therapy.

# PO7-02-YI
# Erodosteine attenuates metabolic dysfunction-associated fatty liver disease in experimental animals: molecular pathway insight

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**Background and aims:** Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by accumulation of fat in the hepatocytes, in the absence of other causes. Erdosteine (Erdo), a thiol based medicine, is recognized to have important antioxidant and anti-inflammatory characteristics. It reduces reactive oxygen species, inflammation and apoptosis. We have investigated the effect of Erdo on High fatty diet (HFD), methionine and choline deficient (MCD) diet-induced MASLD and metabolic dysfunction-associated steatohepatitis (MASH) in male Sprague Dawley rats

**Method:** To induce MASLD, rats were fed with HFD along with fructose water for 10 weeks whereas MASH was induced by feeding MCD diet for 42 days. Before using the test drug, both the animal models were standardized in our lab. In the animal model of MASLD, Erdo (2.5, 5, 10 mg/kg) administered for the last four weeks of diet administration protocol. The optimum dose was further evaluated for in animal model of MASH

**Results:** MASLD and MASH was successfully induced as indicated by Haematoxylin and eosin staining, Oil red O staining and significant increase in body weight, liver weight, increase in serum AST, ALT and ALP, TG levels and LDL, with decreasing HDL cholesterol levels. Erdo at the dose of 10mg/kg most effectively attenuated high fatty diet induced MAFLD and MASH by reducing oxidative stress, inflammation and apoptosis. Erdo at the highest dose (10mg/kg) preserved the morphological structure of liver tissue. On analysis of the inflammatory MAPK pathway, we found that the expression of the pro-survival kinase ERK 1⁄2 was higher while JNK and p38 protein expressions were lower with erdo treatment. Erdo at a dose of 10 mg/kg significantly reduced oxidative stress and inflammation by decreasing the levels of pro-inflammatory cytokines (TNF, IL-6, IL-1Beta, )

**Conclusion:** Erdo has promising therapeutic potential in treatment of MASLD and MASH, owing to its anti-inflammatory, anti-oxidant properties and anti-apoptotic properties.

**Figure: Gross liver appearance of rats in different experimental groups**

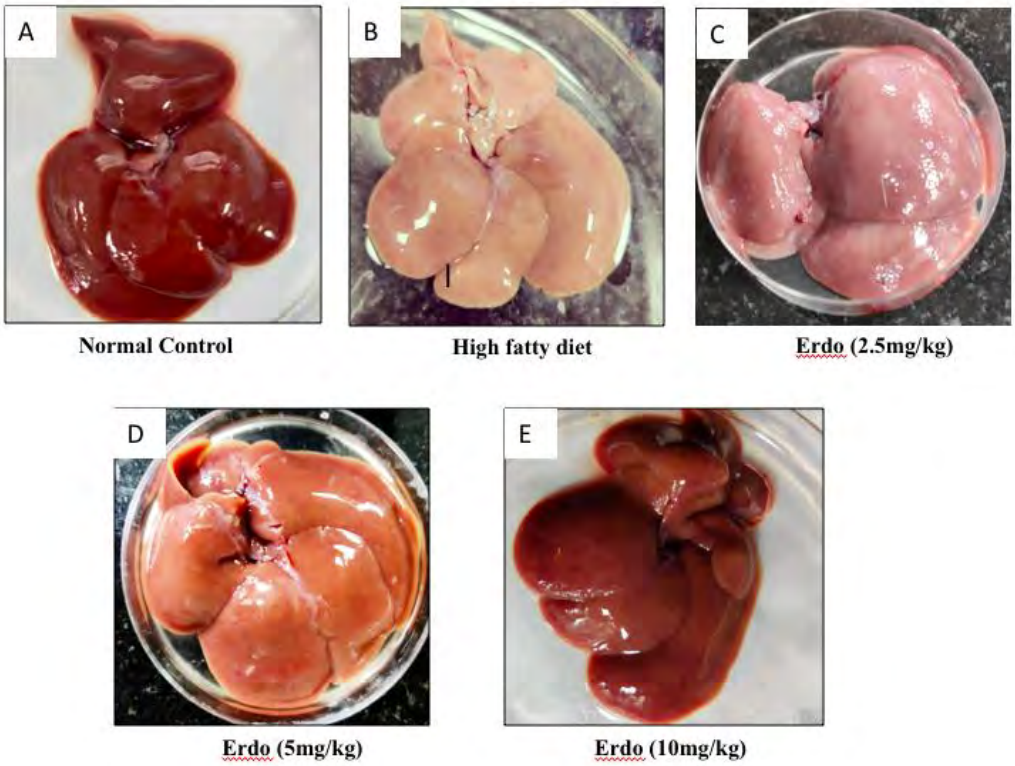


**Figure 2. Effect of Erdo on histopathological changes in liver tissue by using HandE staining.**

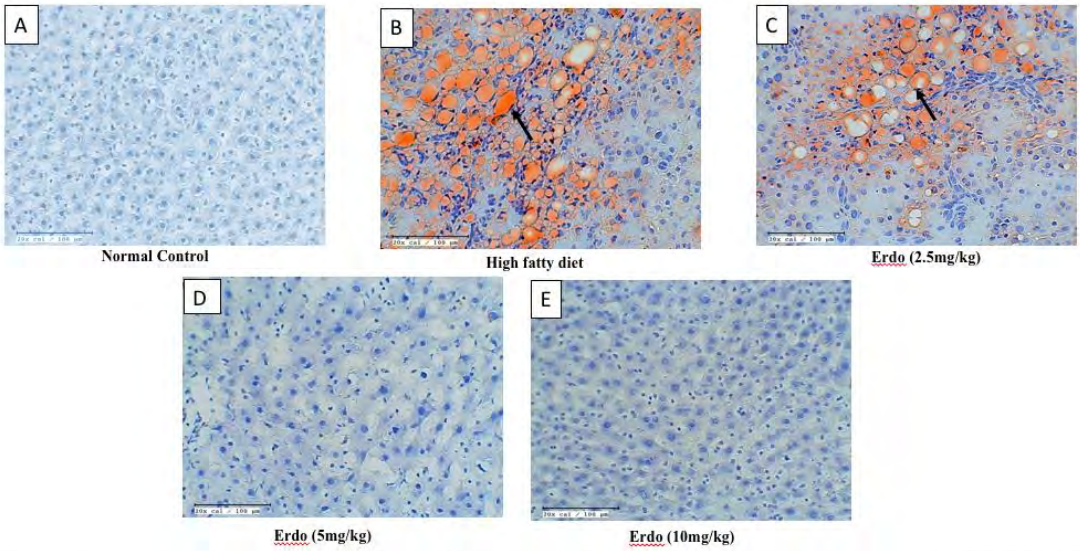


Fig: A show normal liver architecture of liver; parenchyma (Controls) without significant Oil Red stain positive steatosis; B and C show sections of liver with macro vesicular fat vacuoles stained with Oil red o stain (arrows). D and E show normal liver parenchyma) without significant Oil Red stain positive steatosis (D-E 10x)

**Figure 3. Effect of Erdo on histopathological changes in liver tissue by using Oil red O staining.**

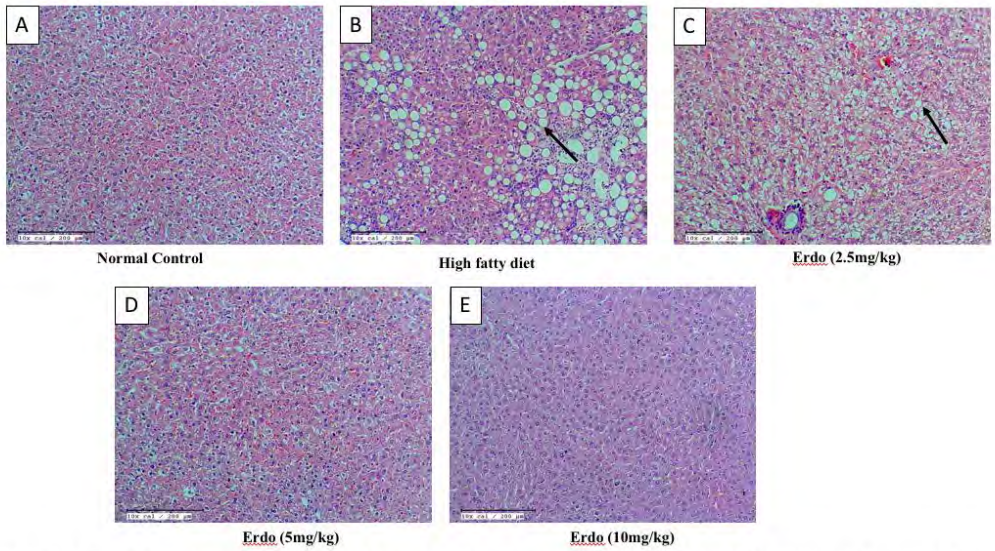


**Figure A: show normal liver parenchyma; Figure B, C show variable macro vesicular steatosis, the steatosis (arrow) is associated with features of chronic hepatitis; In Fig. C, steatosis is mainly seen in Zone 3 and 2 of liver (arrow).g. Figures D show normal liver parenchyma without significant inflammation or steatosis; Figure E: also show normal architecture of liver**

**Table 1. Effect of Erdo on oxidative stress and inflammatory markers:**

| Groups | Malondialdehyde (nmoles/g of tissue) | Reduced glutathione (umoles/g of tissue) | Superoxide dismutase (U/mg of protein) |
|---|---|---|---|
| N.C. group | 25.83 ± 1.85 | 0.29 ± 0.01 | 6.34 ± 0.26 |
| Disease Control group | 55.36 ± 1.28 | 0.06 ± 0.02*** | 2.96 ± 0.18*** |
| Erdo (2.5mg/kg) | 48 ± 2.41 | 0.09 ± 0.02 | 3.01 ± 0.2 |
| Erdo (5mg/kg) | 41 ± 2.21 | 0.16 ± 0.04 | 3.9 ± 0.31 |
| Erdo (10mg/kg) | 33 ± 3.95### | 0.203 ± 0.01### | 4.82 ± 0.28### |

The values are expressed as mean ± S.E.M. n = 6 in each group. *** p <0.001 versus normal control, ###
p <0.001 versus Disease group.

# PO7-05
# Prognostic value of ultra-low-pass whole genome sequencing of circulating tumor DNA in hepatocellular carcinoma under systemic treatment

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**Background and aims:** New prognostic markers are needed to identify patients with hepatocellular carcinoma (HCC) who carry a worse prognosis. Ultra-low-pass whole-genome sequencing (ULP-WGS) (≤0.5× coverage) of cell-free DNA (cfDNA) has emerged as a low-cost promising tool to assess both circulating tumor DNA (ctDNA) fraction and large structural genomic alterations. We studied the performance of ULP-WGS of plasma cfDNA to infer prognosis in patients with HCC.

**Method:** Plasma samples were obtained from patients with HCC prior to surgery, locoregional or systemic therapy, and were analyzed by ULP-WGS of cfDNA to an average genome-wide fold coverage of 0.3x. ctDNA and copy number alterations (CNA) were estimated using the software package ichorCNA.

**Results:** Samples were obtained from 73 HCC patients at different BCLC stages (BCLC 0/A: n = 37, 50.7%; BCLC B/C: n = 36, 49.3%). ctDNA was detected in 18 out of 31 patients who received systemic treatment. Patients with detectable ctDNA showed significantly worse overall survival (median, 13.96 months vs not reached). ctDNA remained an independent predictor of prognosis after adjustment by clinical-pathologic features and type of systemic treatment (hazard ratio 7.69; 95%, CI 2.09-28.27). Among ctDNA-positive patients under systemic treatments, the loss of large genomic regions in 5q and 16q arms was independently associated with a poorer prognosis, as indicated by hazard ratios of 8.92 (95% CI, 1.79-44.38) and 5.29 (95% CI, 1.24-22.50), respectively, following multivariate analysis. In ctDNA-positive patients, more than 7 CNA (either gains and losses) was associated with inferior OS (LogRank p value of 0.006). Median OS was 54.63 months (95%CI 21.06-88.21) in the less than 7 CNA group, while it was 10.54 months (95%CI 3.28-17.80) in the more than 7 CNA group.

**Conclusion:** ULP-WGS of cfDNA provides clinically relevant information about the tumor biology. The presence of ctDNA and the loss of 5q and 16q arms in ctDNA-positive patients are independent predictors of worse prognosis in patients with advanced HCC under systemic therapy.

# PO8-03-YI
# High viscoelasticity promotes the progression of hepatocellular carcinoma (HCC) in pre-cirrhotic livers

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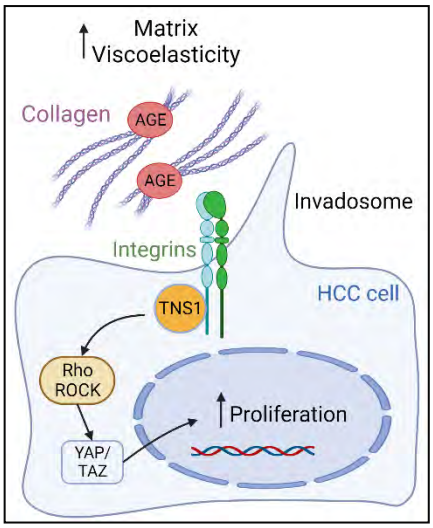
**Background and aims:** The incidence of MASH-related HCC is rapidly increasing. Matrix stiffness is linked to HCC in cirrhosis, however, 30% of tumors occur in non-cirrhotic MASH with low stiffness. In patients with MASH/Type 2 diabetes mellitus (T2DM) advanced glycation end products (AGEs) may create profound changes in the extracellular matrix (ECM). We hypothesized that architectural changes in the collagen network caused by AGEs form a niche that promote HCC invasion.

**Method:** AGEs were studied in healthy subjects and patients with MASH or MASH/T2DM (F1-2). Matrix mechanical properties were tested by atomic force microscopy (AFM) and rheometry. The effects of AGEs were tested in mice on chow, fast food (FFD) or high AGEs diet (HiAD) with/without pyridoxamine (inhibitor of AGE formation), or alagebrium (inhibitor of AGE crosslinking). Receptor for AGEs hepatocyte KO ($RAGE^{hepKO}$) or AGEs clearance receptor (AGER1) transgenic mice were studied. For HCC, hydrodynamic injections (HDI) with hMet/mutant-beta-catenin-myc tag or control were performed. The number of foci was assessed by GS/myc-tag. AFM and rheometry evaluated the mechanical properties. RNAseq analyses were employed to study mechanosignaling. To interrogate YAP/TAZ, we delivered dnTEAD2 vs. control by HDI in the HiAD/HCC model. Tensin1 (TNS1, mechano-transducer) was studied in vivo in a CrisprCas9 KD system. We employed an interpenetrating network-alginate hydrogels (rBM-IPN) with tunable viscoelasticity to assess invasion, TNS1, YAP/TAZ and proliferation.

**Results:** Matrix AGEs were linked to higher viscoelasticity in MASH/T2DM while stiffness was similar in patients. Liver AGEs significantly increased in the HiAD group compared to those on FFD ($p < 0.001$), and tumor foci appeared earlier, and progression was faster. Pyridoxamine and alagebrium reduced viscoelasticity and the number of tumor foci ($p < 0.001$). Similar results were seen in the $RAGE^{hepKo}$, and AGER1 transgenic mice with lower AGEs. RNAseq data depicted Hippo pathway activation in the HiAD group, that was AGEs-dependent. This was confirmed by active nuclear YAP. The effects on tumor progression were reversed by dnTEAD. In 3D hydrogels, the AGEs crosslinked network had similar stiffness, but architectural changes with bundling, lower interconnectivity, and significantly increased viscoelasticity promoting YAP activation, proliferation and invadopodia formation in integrin-beta1 and TNS1-dependent manner. TNS1 KD reduced the number of tumor foci as well as the expression of YAP target genes in the HiAD/HCC group.

**Conclusion:** Matrix accumulation of AGEs in MASH/T2DM alters the collagen network with lower interconnectivity, creating a niche with high viscoelasticity that promotes HCC progression trough integrin beta1 and TNS1 mechanosignaling. Increased viscoelasticity in pre-cirrhotic livers could be an important biomarker for HCC risk.

**Figure:**


Matrix
Viscoelasticity
Collagen
AGE
AGE
Invadosome
Integrins
HCC cell
TNS1
Rho
ROCK
Proliferation
YAP/
TAZ

# PO8-11-YI
# A novel muti-tactic approach to block immune checkpoints PD-1/PD-L1 and CD155/TIGIT in hepatocellular carcinoma using an array of non-coding RNAs

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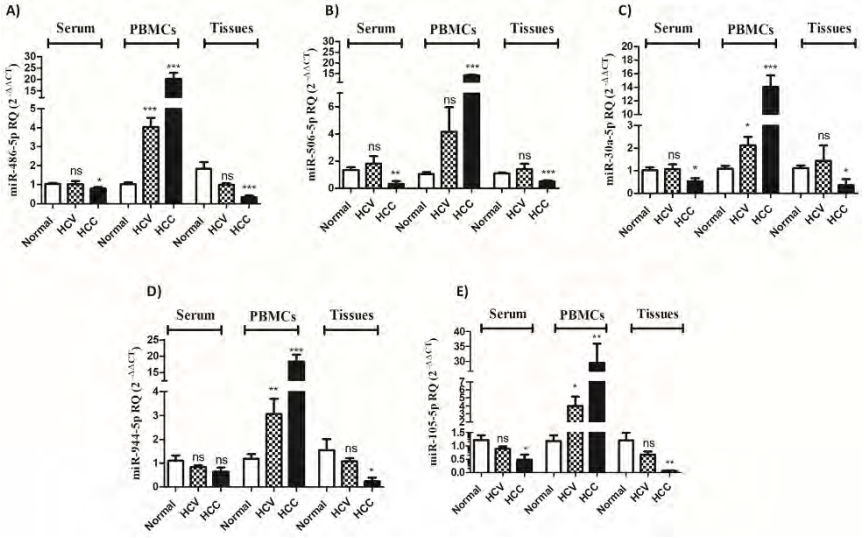
**Background and aims:** Tumor microenvironment (TME) is an intricate web of stromal and immune cells creating an immune suppressive cordon around the tumor. In hepatocellular carcinoma (HCC), TME is a formidable barrier towards novel immune therapeutic approaches evading the oncology field. In this study, the main aim was to identify the intricate immune evasion tactics mediated by HCC cells and to study the epigenetic modulation of the immune checkpoints; PD-1/PD-L1 and TIGIT/CD155at the tumor-immune synapse.

**Method:** Liver biopsies and whole blood were collected from Hepatitis C Virus (HCV) (n = 35), HCC (n = 45) and healthy individuals (n = 20). PBMCs and sera were isolated from whole blood using the Ficoll-density gradient centrifugation method. PBMCs and Huh7 cells were cultured and transfected with an array of oligonucleotides. Total RNA was extracted and quantified by qRT-PCR. Co-culturing of Huh7 cells and PBMCs isolated from HCC patients was performed with an effector-to-target 5:1. In vitro cytolytic activity of PBMCs of HCC patients and healthy controls against their target hepatocytes was assessed using the LDH Assay.

**Results:** Screening was performed to PD-L1/PD-1 and CD155/TIGIT axes in HCC patients. PDL1, CD155, PD-1 and TIGIT were found to be significantly upregulated in liver tissues and PBMCs of HCC patients. An array of lncRNAs and microRNAs validated to regulate such immune checkpoints were screened. The lncRNAs; CCAT-1, H19, and MALAT-1 were all significantly upregulated in the sera, PBMCs, and tissues of HCC patients as compared to HCV patients and healthy controls. However, miR-944-5p, miR-105-5p, miR-486-5p, miR-506-5p, and miR-30a-5p were downregulated in the sera and liver tissues of HCC patients. On the target tumor cell side, knocking down of all three lncRNAs—CCAT-1, MALAT-1, or H19—markedly decreased the co-expression of PD-L1 and CD155 and accordingly induced the cytotoxicity of co-cultured immune cells. On the immune effector side, ectopic expression of the under-expressed microRNAs; miR-486-5p, miR-506-5p, and miR-30a-5p significantly decreased the transcript levels of PD-1 in PBMCs with no effect on TIGIT. On the other hand, ectopic expression of miR-944-5p and miR-105-5p in PBMCs dramatically reduced the co-expression of PD-1 and TIGIT. Finally, all studied miRNAs enhanced the cytotoxic effects of PBMCs against Huh7 cells. However, miR-105-5p showed the highest augmentation for PBMCs cytotoxicity against HCC cells.

**Conclusion:** This study highlights a novel co-targeting strategy using miR-105-5p mimics, MALAT-1, CCAT-1 and H19 siRNAs to efficiently hampers the immune checkpoints; PD-L1/PD-1 and CD155/TIGIT immune evasion properties in HCC.

**Figure:**


A)
Serum
PBMCs
Tissues
miR-486-5p RQ (2^-ΔΔCT)
B)
miR-506-5p RQ (2^-ΔΔCT)
C)
miR-30a-5p RQ (2^-ΔΔCT)
D)
miR-944-5p RQ (2^-ΔΔCT)
E)
miR-105-5p RQ (2^-ΔΔCT)
Normal
HCV
HCC
ns

# PO8-12
# Atezolizumab-targeted liposomal doxorubicin modulates PD-L1 expression and reduces the invasive phenotype in in vitro models of hepatocellular carcinoma

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**Background and aims:** Hepatocellular carcinoma (HCC) is the primary cancer of the liver and, despite the novel therapeutic options available, including the combo atezolizumab/bevacizumab, is still characterized by elevated morbidity and mortality, and effective therapies or combinations remain an unmet medical need. We have obtained promising results regarding the cytotoxic activity of atezolizumab targeted liposomal doxorubicin (DXR) in preclinical models of HCC. To gain new insights on the mechanism of PD-L1 targeted liposomal DXR, we investigated its effect on the phenotype of liver cancer cells and macrophages, as well as on epithelial-to-mesenchymal (EMT) transition, using untargeted liposomal DXR as a control, in 2D and 3D cellular HCC models.

**Method:** INF-g was used to induce PD-L1 overexpression in HepG2 2D cultures and spheroids. A spheroid with HepG2 and the monocytic cells THP-1 was set up to assess the effect of atezolizumab-targeted liposomal DXR (Stealth Immunoliposomes, SIL) and its untargeted counterpart (Stealth Liposomes, SL) on PD-L1 expression, macrophage polarization and clonogenicity and invasiveness of tumor cells. The effect of SL and SIL on the EMT process was evaluated on the epithelial marker E-cadherin and the mesenchymal marker Vimentin on TNF-α-treated HepG2 cells.

**Results:** Only SIL was able to decrease the INF-g-induced PD-L1 overexpression on HepG2 2D cultures and spheroids ($p < 0.01$ and $p < 0.001$ respectively). In THP-1/HepG2 spheroids, although both SL and SIL decreased clonogenicity and invasiveness of HepG2 cells ($p < 0.0001$) and reduced pro-tumoral CD-163-expressing macrophages ($p < 0.0001$), only SIL decreased PD-L1 expression ($p < 0.01$), confirming its peculiar immunomodulatory activity. Accordingly, SIL significantly increased E-cadherin expression and decreased Vimentin one, downregulated and upregulated by TNF-α, respectively, at variance to SL ($p < 0.001$), suggesting the SIL role in decreasing EMT. Furthermore, SIL decreased PD-L1 expression also in the TNF-α model ($p < 0.001$ vs SL).

**Conclusion:** Atezolizumab targeted liposomal DXR modulates the invasive phenotype of HCC cells and macrophage polarization, thereby exerting a complex effect on the tumor immune microenvironment. These preliminary in vitro results shed new light on the mechanism of this nanomedicine, which deserves further in vivo validation.

# PO8-14-YI
# Implications and therapeutic potential of neddylation for pediatric liver cancer: hepatoblastoma

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*[1]Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Liver Disease Lab, Spain*, *[2]Mendel University in Brno, Department of Chemistry and Biochemistry, Czech Republic*, *[3]Carlos III National Health Institute, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Spain*, *[4]Germans Trias i Pujol Research Institute (IGTP), Childhood Liver Oncology Group, Program for Predictive and Personalized Medicine of Cancer (PMPPC), Spain*, *[5]Univ. Montpellier, CRBM, CNRS, France*, *[6]University of Salamanca, IBSAL, Experimental Hepatology and Drug Targeting (HEVEFARM), Spain*, *[7]University of Navarra, Hepatology Program, CIMA, Spain*

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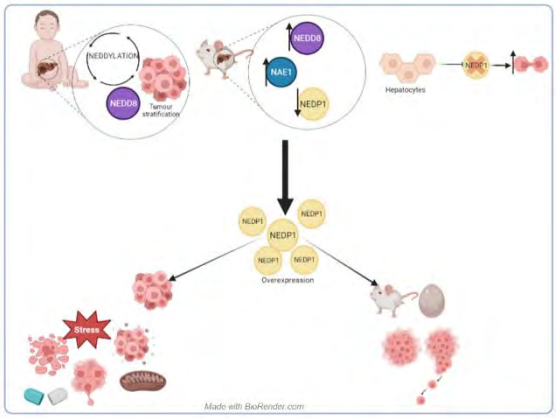
**Background and aims:** Hepatoblastoma (HB) is a rare type of primary liver cancer that mainly affects children. Currently, the main treatment options include surgical resection accompanied by chemotherapeutics such as cisplatin or doxorubicin. However, these options are limited or ineffective due to poor prognosis, high recurrence rate and significant side effects. This is a tumor with a low rate of somatic mutations, in which the involvement of other mechanisms modulating its tumorigenic capacity stands out. In this regard, it is crucial to highlight the adaptive advantages conferred to the tumor by post-translational modifications. Neddylation has been extensively studied in this context. Based on this evidence, we have considered analyzing the impact that neddylation could have on the development of HB.

**Method:** A cohort of HB patients, preclinical animal model and *in vitro* model in tumour cells were used to characterise NEDDylation pathway in HB. Besides the modulation of NEDP1 levels using an *in vitro* approach was made to study cell proliferation, migration, and metabolic status. *In vivo*, the implications of NEDP1 overexpression as tumour suppressor was evaluated.

**Results:** Transcriptomic analysis in samples from patients with HB has demonstrated an increase in the neddylation cycle. Likewise, in preclinical models of HB, both *in vivo* and *in vitro*, we have identified an increase in NEDD8 and NAE1 (the NEDD8-activating enzyme E1), which is related to an increase in global neddylation. A significant reduction in the levels and activity of NEDP1 deneddylases has also been observed, which justifies the importance of this process in the development and progression of this pathology. In addition, a positive impact on proliferation driven by neddylation is evidenced. The use of pharmacological inhibitors of neddylation has been shown to decrease such proliferation, while the increase in global neddylation induced by NEDP1 inhibition has resulted in its increase. Moreover, modulation of Neddylation through increased levels of NEDP1, *in vitro* and *in vivo* approaches, reveals induction of apoptosis, modulation of migratory and proliferative capacity and metabolic reprogramming, both in HB cell lines (HepT1 and HepG2) and in patient-derived xenografts (PDX) from distal metastasis. *In vivo*, *in Ovo* and *ex Ovo* experiments showed a reduction of tumorigenicity and metastatic phenotype; mice models of HB showed, at the histological level, a reduction of proliferation and tumorigenesis, as well as an increase of necrosis, and a reorganization of the proteome, with an increase of apoptotic processes.

**Conclusion:** The effect observed with NEDP 1 overexpression points to the importance of post translational modifications in pathologies such as HB and highlights the relevance of NEDDylation, not only in the molecular characterization of HB, but also in the development of new specific treatments.

**Figure:**


NEDDYLATION
NEDD8
Tumour stratification
NEDD8
NAE1
NEDP1
Hepatocytes
NEDP1
NEDP1
NEDP1
NEDP1
NEDP1
NEDP1
Overexpression
Stress
Made with BioRender.com

# PO8-15
# Hepatitis B virus-driven intercellular crosstalk and telomere maintenance in hepatocellular carcinoma

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**Background and aims:** Liver fibrosis and cirrhosis progression can be accelerated by hepatic stellate cell (HSC) activation and promote hepatocellular carcinoma (HCC) occurrence. This research aimed to reveal the role and mechanism of HBV-induced hepatocyte-HSC crosstalk and its contribution to liver fibrosis and HCC development.

**Method:** Single-cell RNA sequencing (scRNA-seq) was performed on tumor tissues from 10 HCC patients with HBV. The pHBV4.1 plasma was used to perform HBV replication cell models. CRISPR/Cas9 gene knockout cells and *Insr*$^{fl/fl}$; Lrat-Cre mice were generated to achieve specific knockout of the *INSR* gene in HSCs. The involvement of HBV replication in liver fibrosis and HCC progression were investigated *in vivo* using MYC-driven LCSC organoids from murine liver cells.

**Results:** HBV transcription affected hepatocyte development, activated the DNA repair pathway, and promoted glycolysis. Hepatocytes with high HBV transcription interacted with activated HSCs (aHSCs) through the NAMPT (ligand)-INSR (receptor) pathway. HBV activated NAMPT through the DNA damage receptor ATR. NAMPT-INSR-mediated hepatocyte-HSC crosstalk caused HSCs to develop a myofibroblast phenotype and activated telomere maintenance mechanisms (TMMs) via PARP1 multisite lactylation. Furthermore, in vivo tumor allografts showed that HBV-mediated liver fibrosis and HCC progression were entirely blocked by inhibiting the ATR-NAMPT-INSR-PARP1 pathway through the use of ATR inhibitors, INSR knockout in HSCs, NAMPT knockdown, or PARP1 inhibitors.

**Conclusion:** HSC activation following HBV infection is highly dependent on crosstalk with hepatocytes through activation of the ATR-NAMPT-INSR pathway, which affects telomere maintenance and PARP1 lactylation. Thus, the ATR-NAMPT-INSR pathway represents a promising therapeutic target for HBV-induced liver fibrosis and HCC.

**Figure:**

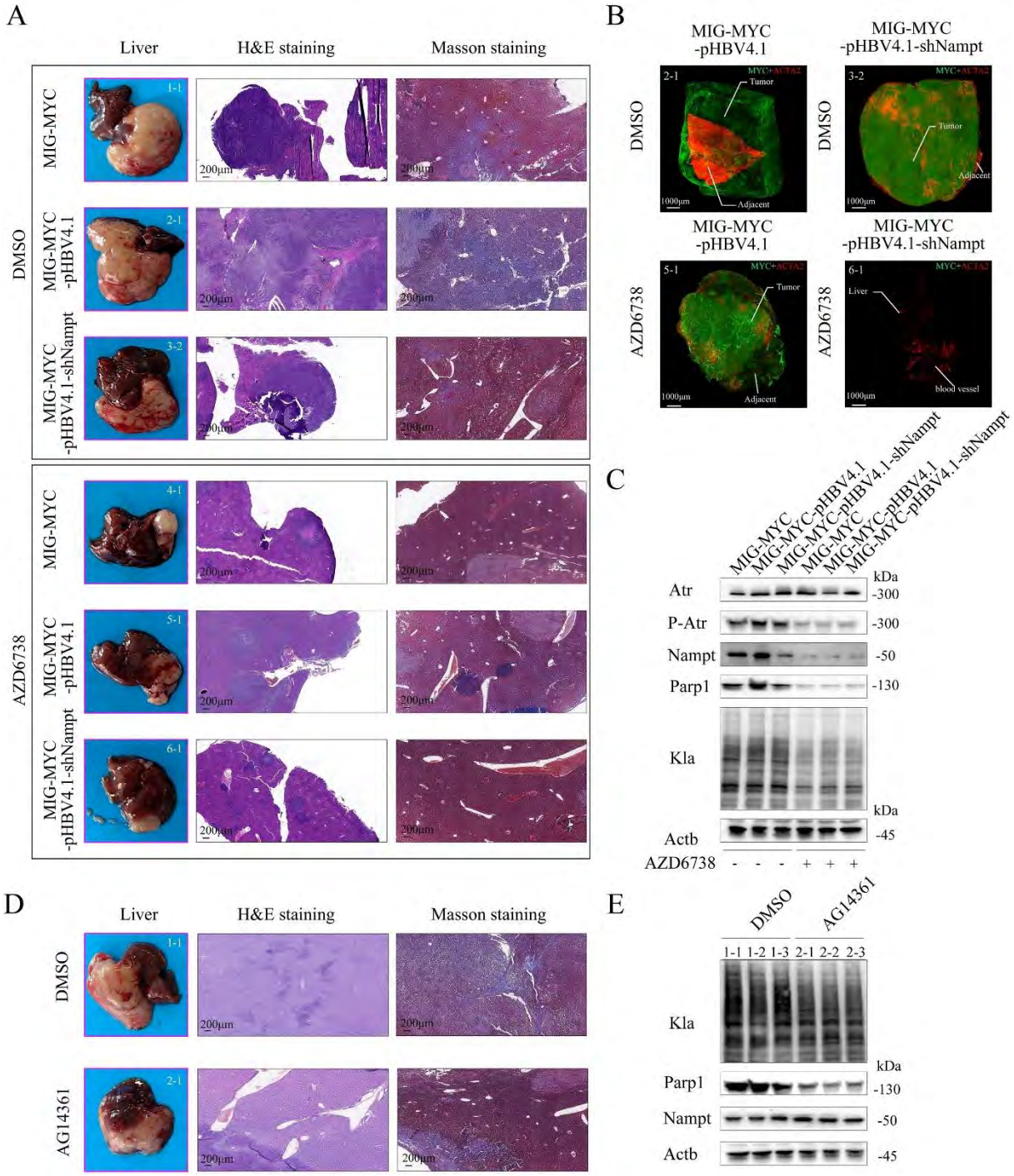
A
Liver
H&E staining
Masson staining
DMSO
AZD6738
MIG-MYC
MIG-MYC -pHBV4.1
MIG-MYC -pHBV4.1-shNampt
1-1
2-1
3-2
4-1
5-1
6-1
200μm
B
MIG-MYC -pHBV4.1
MIG-MYC -pHBV4.1-shNampt
DMSO
AZD6738
MYC+ACTA2
Tumor
Adjacent
Liver
blood vessel
1000μm
C
MIG-MYC
MIG-MYC-pHBV4.1
MIG-MYC-pHBV4.1-shNampt
Atr
P-Atr
Nampt
Parp1
Kla
Actb
kDa
-300
-50
-130
-45
AZD6738
D
Liver
H&E staining
Masson staining
DMSO
AG14361
E
DMSO
AG14361
1-1 1-2 1-3 2-1 2-2 2-3
Kla
Parp1
Nampt
Actb
kDa
-130
-50
-45

**Fig.7 Effects of ATR-NAMPT pathway mediated PARP1 lactylation on HCC and liver fibrosis in murine oncogene-driven allograft HCC model.**
**(A)** HandE and Masson staining for MIG-MYC, MIG-MYC-pHBV4.1, and MIG-MYC-pHBV4.1-shNampt mice liver following exposure to DMSO or AZD6738 treatments (6 mice in each group). **(B)** Tissue clearing and image analysis from representative MIG-MYC-pHBV4.1 mice, MIG-MYC-pHBV4.1-shNampt mice treated with DMSO (mice 2-1 and 3-2, respectively) or AZD6738 (mice 5-1 and 6-1, respectively): MYC labeled tumor cells were stained green, whereas ACTA2 positive cells were stained red. **(C)** Western blot for Atr, P-Atr, Nampt, Parp1, and lactylation levels from MIG-MYC, MIG-MYC-pHBV4.1, and MIG-MYC-pHBV4.1-shNampt mice liver following exposure to DMSO or AZD6738 treatments. **(D)** HandE and Masson staining, and **(E)** Western blot for lactylation, Parp1, and Nampt levels, from MIG-MYC-pHBV4.1 mice liver following exposure to DMSO or AG14361 treatments.

# POSTER ABSTRACT PRESENTATIONS

# Clinical Science

# PO1-01
# Utility of liver biopsy in assessment of small hepatic focal lesions in cirrhotic patients : a single center experience

Mohamed Fathey Abdelkhalek Elgazzar[1], Mohamed Kohla[1], Dina sweed[1], mohamed abo naem[2], anwar anwar mohamed[1]

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**Background and aims:** Hepatocellular carcinoma (HCC) is a rapidly fatal cancer, overall, one third of cirrhotic patients will develop HCC during their Life time. Diagnosis of small HCC lesions lacking typical hemodynamic criteria (arterial enhancement, portal and delayed washout) could be a challenge. Atypical enhancement patterns seen in a considerable number of HCC patients have led to around 35% false negative results in patients with tumors between 1-2 cm in diameter in triphasic computed tomography (CT) scan. The differential diagnosis among these lesions constitutes a major task. One of the main difficulties in liver imaging for metastatic disease is the high prevalence of benign liver lesions that can be misinterpreted as evidence of metastatic disease, thus dramatically changing a patients stage, and therefore treatment options. Therefore, an understanding of the various appearances of metastatic disease is crucial. Lesion in a cirrhotic patient that lacks typical imaging characteristics, histopathological evaluation is the recommended diagnostic tool. We aimed is to know the histological pattern of atypical hepatic focal lesions and to recognize whether it is a benign or malignant conditions.

**Method:** This study is a retrospective study that was done on liver biopsy from hepatic focal lesions without HCC criteria on radiological investigation. All patients attended the HCC clinic of NLI, Menoufia university, Egypt with small hepatic focal lesion (less than 3 cm) and without HCC criteria by triphasic CT scan and dynamic MRI from 2015 to 2021

**Results:** 108 Biopsy samples from 108 atypical HFLs without HCC criteria in triphasic CT nor dynamic criteria stained with Haematoxylin and Eosin and examined by expert pathologists. 43 patients (39.8%) of the lesions showed HCC patterns, 6 patients (5.6%) were cholangiocarcinoma, 34 patients (31.5%) were metastatic lesions, the most common metastsis was colorectal which constitutes 15 patients (44.1%). Pancreatic carcinoma metastasis was 10 patients (29.4%) of the metastatic lesions followed by lung cancer metastasis 3 patients (8.8%). 25 patients (23.2%) were benign. Dysplastic nodules were the most common benign atypical HFL 16 patients (14.8%), haemangioma seen in 3 lesions (2.7%) and focal nodular hyperplasia seen in 2 lesions (1.8%). AFP, ALT and AST were significantly higher ($p < 0.05$) in patients with atypical HCC lesions than in patients with benign lesions.

**Conclusion:** In our study HCC was the most common atypical HFL. Colorectal metastasis was the most common metastatic lesion followed by pancreatic metastasis and then lung cancer metastasis. Dysplastic nodules were the most common benign lesions followed by haemangioma and then focal nodular hyperplasia. AFP, ALT and AST were significantly higher in HCC patients with atypical radiological pattern than in patients with benign lesions.

# PO1-02-YI
# Circulating hypermethylated RASSF1A as a marker of hepatocellular carcinoma in chronic HCV patients

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**Background and aims:** Hepatocellular carcinoma (HCC) is a global health problem and the fourth leading cause of cancer related deaths worldwide. HCC is typically undistinguished initially causing detection of the majority of cases in palliative stages. Thus, in this study, we aimed to assess the role of plasma level of methylated RASSF1A in post hepatitis C virus, (HCV) cirrhotic patients as a non-invasive diagnostic marker for HCC.

**Method:** The levels of methylated ras association domain family 1 isoform A (RASSF1A) were determined in plasma of 120 participants who were classified to three groups (40 participants each). HCC patients on top of chronic HCV infection, post HCV cirrhotic patients without HCC in addition to healthy matched age and sex control group. Methylation sensitive restriction enzyme digestion and real-time quantitative polymerase chain reaction method were used to measure the expression level of RASSF1A.

**Results:** Patients with HCC exhibited significantly higher levels of circulating hypermethylated RASSF1A than post hepatitis C patients and control groups. In addition, a statistically significant correlation between RASSF1A and the different clinic pathological parameters was observed. The receiver operating characteristic curves plotted showed that plasma RASSF1A helped in significant differentiation between HCC and cirrhotic patients.

**Conclusion:** Circulating hypermethylated RASSF1A could be used as a non-invasive diagnostic marker for discriminating HCC among post HCV cirrhotic patients and characterizing their progression.

# PO1-03-YI
# Evaluation of glycemia and diabetes as prognostic factors in advanced hepatocellular carcinoma treated with systemic therapy

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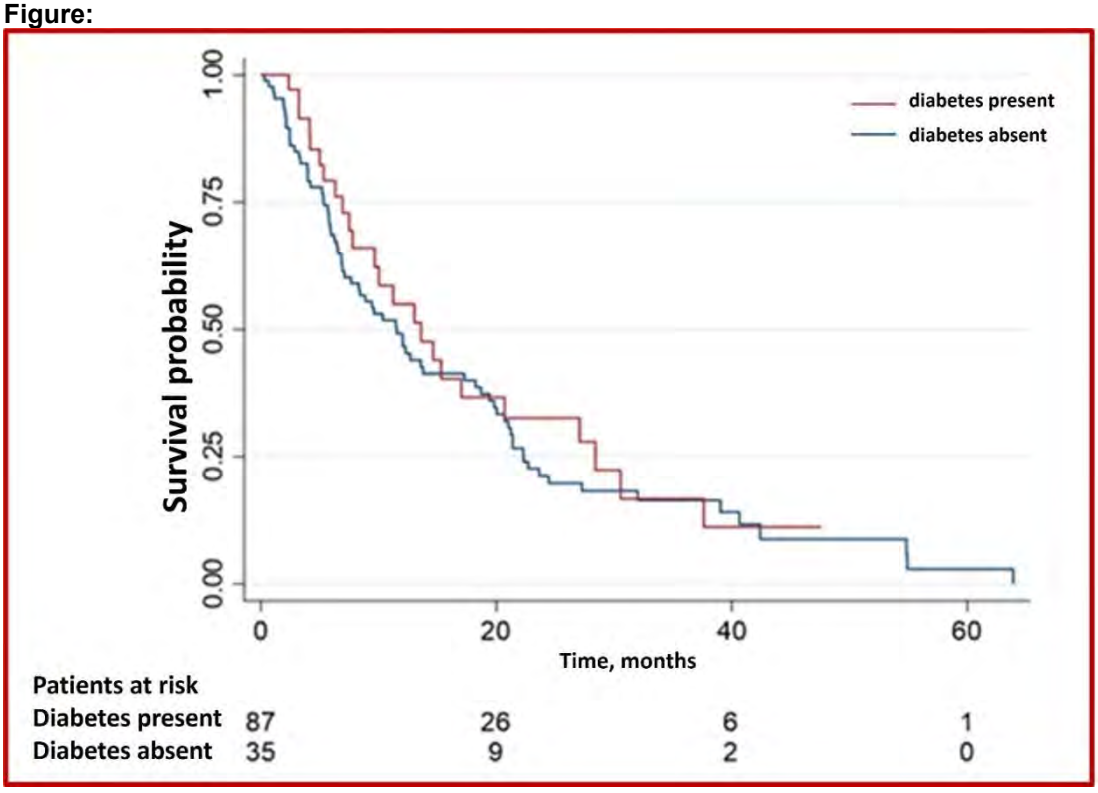
**Background and aims:** Hepatocellular carcinoma (HCC) patients with coexisting diabetes mellitus (DM) have a shorter survival time and a higher risk for tumor recurrence after curative treatments. Systemic therapy (ST) of advanced HCC (aHCC) involves the use of tyrosine kinase inhibitors or immunotherapy, as recommended by European guidelines and the Barcelona Clinic Liver Cancer (BCLC) classification. Here, we investigated the prognostic role of glycemia and DM in patients with aHCC treated with ST. Furthermore, we searched for other possible prognostic factors among clinical or laboratory findings. Finally, we assessed the prevalence of the main adverse events (AEs) to treatment.

**Method:** In this retrospective, monocentric study we enrolled N = 128 consecutive patients affected by aHCC, treated with first-line ST (sorafenib, lenvatinib, atezolizumab/bevacizumab, or other drugs in clinical trials), between March 2008 and September 2023. N = 3 patients were lost at follow-up (FU). Clinical and laboratory data were collected at HCC diagnosis, treatment start and treatment suspension (or at last FU visit for those with ongoing treatment). For the statistical analysis, all patients were re-classified according to the 2022 BCLC prognostic stratification.

**Results:** During the FU period, 100 patients died, none underwent liver transplantation, with an incidence rate of death of 0.06 per month and a median survival of 12.0 months (IQR 5.7-22.7). Among the enrolled patients, N = 36 (29%) had DM as known comorbidity at HCC diagnosis. A fasting glycemia level at the start of ST was available in N = 115 patients; among these, N = 22 had a glycemia level ≥126 mg/dL. No statistical association between DM and overall survival (OS) was observed (rate ratio 0.830, 95% CI 0.523-1.316, p = 0.427; **Figure 1**). Similarly, our study found no statistical association between increased glycemia levels (≥126 mg/dL) at ST start and OS (rate ratio 0.752, 95% CI 0.438-1.291, p = 0.300). At a Cox proportional hazards model, the laboratory parameters shown to be independent predictors of early mortality were the following: increased alanine aminotransferase (ALT) (≥2 × the upper normal limit) and total bilirubin (≥1.5 mg/dL), platelet count ≥60, 000/uL, hemoglobin ≥10 g/L, and neutrophil/lymphocyte ratio (NLR) ≥3. The main AEs observed, frequently implied in temporary or definitive suspension of treatment, were hand-foot syndrome, fatigue, anorexia, diarrhea, and altered liver biochemistry. Hyperglycemia never brought to definitive withdrawal of therapy.

**Conclusion:** Our study suggests the lack of an association between hyperglycemia and/or DM and OS among aHCC patients who undergo ST, which is not hampered by coexistent DM/hyperglycemia. Other laboratory findings universally available in clinical practice, such as ALT levels and NLR, were associated with poorer prognosis in the study population.

**Figure:**


diabetes present
diabetes absent
Survival probability
1.00
0.75
0.50
0.25
0.00
0
20
40
60
Time, months
Patients at risk
Diabetes present 87 26 6 1
Diabetes absent 35 9 2 0

# PO1-04-YI
# Improved handling of BCLC 2022 update in the management of hepatocellular carcinoma in clinical practice

Eleonora Alimenti[1], Massimo Iavarone[1], Lorenzo Canova[1 2], Mariangela Bruccoleri[1], Barbara Antonelli[3], Anna Maria Ierardi[4], Angelo Sangiovanni[1], Pietro Lampertico[1 5]

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**Background and aims:** In 2022 the BCLC staging and treatment algorithm for hepatocellular carcinoma (HCC) management has been updated, granting for higher flexibility and customized management with a multidisciplinary decision process in the choice of the therapeutic strategy for HCC patients as compared to the 2018 version.

To evaluate the ability to adhere to BCLC 2022 in daily clinical practice as compared to the BCLC 2018 and the impact on patients' overall survival (OS).

**Method:** We retrospectively evaluated data of 806 prospectively enrolled (year 2006 to 2022) patients with de novo HCC (Table 1) in different stages according to BCLC: 571 0-A, 133 B, 85 C and 17. First line treatment allocations were discussed by a multidisciplinary team (MTD), following updated recommendations and guidelines. All patients were followed until death or end of follow-up.

**Results:** Overall, the adherence to BCLC increased from 50% for the BCLC 2018 to 68% for the BCLC 2022 ($p < 0.001$) considering all stages of HCC: 48% vs 71% stage 0/A ($p < 0.001$), 50% vs 61% stage B ($p = 0.06$), 59% vs 59% stage C ($p$ = NS), 74% vs 74% for stage D ($p$ = NS). Among BCLC 0/A patients, 131 (32%) were treated with TACE, which is the second choice of treatment according to BCLC 2022, while among BCLC B patients, 5 (6%) underwent LT and 10 (12%) were treated with systemic therapy, which are now considered treatment options for this stage in selected patients. The expected survival rate following the BCLC 2018 recommendations remains unchanged by adhering to the BCLC 2022 update [74.5% vs 75.2% at year 2 ($p$ = NS) and 41.4% vs 44.6% at year 5 ($p$ = NS), respectively]. Finally, the rate of "upward stage migration" was similar by BCLC 2022 or BCLC 2018 (12% vs 14%, $p$ = ns), while the rate of "downward stage migration" was lower with the new one (19% vs 36%, $p < 0.001$). Overall, the 2-year survival rate of patients treated outside the BCLC 2022 recommendations did not significantly differ from those treated according to the updated algorithm (76% vs 74.5%, $p$ = NS). In BCLC B and BCLC C, an upward stage migration was associated to higher rates of 2-year survival (93.5% vs 60.6%, $p = 0.002$ for BCLC B and 36.9% vs 27.4%, $p = 0.01$ for BCLC C), while there was no difference for stage 0/A between those treated outside and those treated according to the BCLC 2022.

**Conclusion:** The BCLC 2022 updated version of HCC staging and treatment system allowed a greater adherence to the algorithm in clinical practice, mainly in the early stages, without adversely affecting the survival of patients. In the intermediate and advanced stages, the access to more radical treatment could offer a survival benefit.

**Figure:**

**Table 1: Characteristics of patients included in the study.**

| Variable | Included patients N=806 |
|---|---|
| Age, years* | 68 (60-74) |
| Born males | 602 (75%) |
| Etiology | |
| HCV | 470 (58%) |
| HBV | 76 (9%) |
| HCV+HBV | 22 (3%) |
| HDV | 19 (2%) |
| Mixed | 36 (4%) |
| Non-viral | 183 (24%) |
| Child-Pugh Class | |
| A | 603/776 (78%) |
| B | 161/776 (21%) |
| C | 12/776 (1%) |
| MELD* | 9 (7-10) |
| Varices | 257/782 (33%) |
| BCLC | |
| 0 | 187 (23%) |
| A | 384 (48%) |
| B | 133 (16%) |
| C | 85 (11%) |
| D | 17 (2%) |
| Number of nodules | |
| 1 | 487/802 (61%) |
| 2-3 | 213/802 (26%) |
| >3 | 102/802 (13%) |
| Largest nodule's size (cm)* | 2.5 (1.6-4.0) |
| First line treatment | |
| Liver transplantation | 39 (5%) |
| Resection | 142 (17%) |
| Radiofrequency ablation | 284 (35%) |
| Transarterial chemoembolization | 210 (26%) |
| Tyrosine-kinase inhibitors | 61 (8%) |
| Immunotherapy | 6 (1%) |
| Transarterial radioembolization | 6 (1%) |
| Combined therapy | 25 (3%) |
| Best supportive care | 33 (4%) |

Data are expressed as number (percentage), unless otherwise specified; *median (IQR)

## PO1-05

# Extracellular vesicles size, concentration and different miRNA expression when compared with serum in MASLD: from steatosis to hepatocellular carcinoma

Mario Álvares-da-Silva[1], melina keingenski[2], Larisse Longo[3], anelise pinto[2], bruno basso[2], thalia schmitz[2], adriana pohlmann[4], josé vargas[2], Isabel Veloso Alves Pereira[5], patricia lopez[6], Jose Tadeu Stefano[7], Claudia P. Oliveira[7], Carolina Uribe[8]

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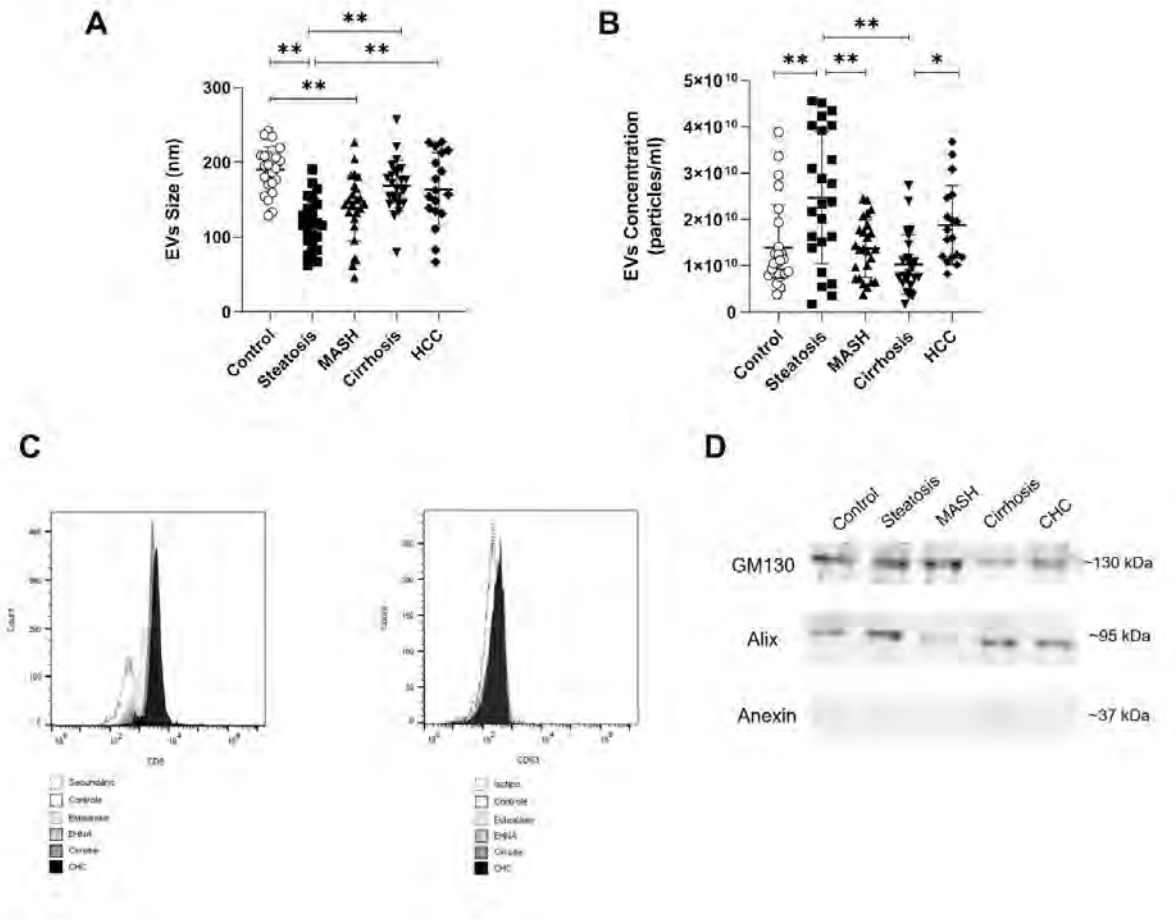
**Background and aims:** Extracellular vesicles (EVs) play a significant role in intercellular communication although their characterization throughout metabolic-dysfunction associated steatotic liver disease (MASLD) and steatohepatitis (MASH) progression still needs to be evaluated. This study aims to characterize EVs in MASLD progression.

**Method:** 167 patients [steatosis (n = 50); MASH (n = 49); cirrhosis (n = 50); and HCC (n = 18)] were evaluated-laboratory plus serum EVs (isolated by size exclusion chromatography and characterized with Nanosight, flow cytometry and Western Blot). miRNAs were evaluated within EVs and in serum.
**Results:** EVs were larger in HCC and cirrhosis in comparison to patients with steatosis ($p < 0.001$), with a higher concentration in HCC vs cirrhosis ($p < 0.05$). For EVs characterization we identified the positive expression of CD9 and CD63 by flow cytometry and Alix and GM130 by Western Blot. There were differences between mirRNA expression in EVs vs. serum. We identified miR-4758 was higher expressed within EVs in steatosis and cirrhosis ($p < 0.05$), while in HCC it was only expressed in the serum. On the other hand, miR-188 and miR-1226 were expressed only in the serum, being absent in EVs ($p < 0.05$). Concerning biochemical variables at each stage of MASLD, AST, GGT, ALP, bilirubin, and creatinine were significantly higher in HCC vs steatosis, MASH and cirrhosis ($p < 0.001$) whereas albumin, TG, TC, WBC and platelets decreased significantly in cirrhosis and HCC vs steatosis and MASH ($p < 0.001$, $p < 0.05$) respectively, caracterizing differences stage and liver function of MASLD.

**Conclusion:** This is the first study to evaluate EVs size and concentration in all MASLD spectrum, showing different EVs size and concentration with MASLD progression, with exosomes in early and microvesicles in advanced stages. Additionally, there are differences between mirRNA expression when compared EVs and serum on MASLD progression. This highlights their association with the progression of MASLD, reinforcing their role as potential biomarkers.

**Figure:**


A
B
C
D
EVs Size (nm)
EVs Concentration (particles/ml)
Control
Steatosis
MASH
Cirrhosis
HCC
GM130
Alix
Anexin
~130 kDa
~95 kDa
~37 kDa

## PO1-06-YI

# Impact of metformin, statin, aspirin and insulin on the prognosis of unresectable HCC patients receiving first line Lenvatinib or Atezolizumab plus Bevacizumab

Elisabeth Amadeo[1], Federico Rossari[1], Margherita Rimini[1], Francesco Vitiello[1], margarida montes[2], Silvia Foti[1], Masatoshi Kudo[3], Toshifumi Tada[4], Goki Suda[5], Shimose Shigeo[6], Sara Leonardi[7], francesca salani[8], Fabian Finkelmeier[9], Lorenzo Antonuzzo[10], Fabio Marra[10], Massiomo Iavarone[11], Giuseppe Cabibbo[12], Francesco Foschio[13], Mario Scartozzi[14], Silvia Camera[1], Mara Persano[14], Takashi Kumada[4], Hideki Iwamoto[6], Atsushi Hiraoka[4], mario scartozzi[14], Luca Aldrighetti[1], Stefano Cascinu[1], Andrea Casadei Gardini[1], José Presa[2]

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**Background and aims:** Recently, in Hepatocellular carcinoma (HCC) setting, the use of metformin has been associated to a trend toward worse response rates, overall survival and progression free survival in patients who received immunotherapy.

**Method:** The study population included individuals from both Eastern and Western regions with a confirmed diagnosis of HCC and receiving first line treatment with atezolizumab plus bevacizumab or lenvatinib. Univariate and multivariate analyses were performed by Cox proportional. For the analysis, patients were stratified based on their use of concomitant medication or not.

**Results:** In the Atezolizumab plus Bevacizumab arm, 50 (16.5%) patients were on chronic metformin use. At the univariate analysis for OS, patients who used metformin showed significantly shorter OS compared to patients who did not (HR 1.9, 95% CI 1.1-3.2). Multivariate analysis confirmed that patients in metformin group had significantly shorter OS compared to patients in no-metformin group (HR 1.9; 95% CI, 1.1-3.1). At the univariate analysis for PFS, patients in metformin group had significantly shorter PFS compared to patients in no-metformin group (HR 1.6, 95% CI 1.0-2.6). Multivariate analysis confirmed that patients in metformin group had significantly shorter PFS compared to patients in no-metformin group (HR 1.7; 95% CI, 1.1-2.7; p = 0.0147). No difference was reported in terms of ORR and DCR between patients in metformin group and those in no-metformin group. In the Lenvatinib cohort, 65 (15%) patients were recorded to chronically use metformin. No statistically significant difference in terms of both OS and PFS was found between patients in metformin group and patients in no-metformin group.

**Conclusion:** This analysis unveils a negative prognostic role associated with metformin use specifically within the Atezolizumab plus Bevacizumab group.

# PO1-10
# Developing a data-driven framework to assess the performance and value of multi-disciplinary teams in HCC

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**Background and aims:** Regular meetings of multi-disciplinary teams (MDTs) constitute a key point of care in the patient care pathway in hepatocellular cancer (HCC). MDTs have been shown to increase the efficiency in health care by increasing number patients treated, improving diagnosis, and positively impacting survival. MDTs are increasing in relevance as the therapeutic landscape evolves towards early-stage treatment and personalization, however there is a lack of methodology for assessing its value and enable continuous improvement. It has also been acknowledged that the way MDTs are organized varies across countries, hospitals and therapeutic areas, which may lead to sub-optimal outcomes. We aim to (1) develop a framework to assess MDT performance tailored to HCC and (2) further enhance it by defining standardized qualitative and quantitative metrics, so that hospitals can assess its maturity and demonstrate the value of MDTs with the goal of sharing cross-centre practices and improve patient outcomes.

**Method:** Using a pre-defined maturity model previously developed for lung cancer (Nadal et al, 2023). We conducted 80 semi-structured interviews with key members of MDTs treating HCC in 14 leading hospitals across Europe and Canada to analyse the MDT process, capabilities, governance, communication channels and its impact on treatment decision-making and patient outcomes. A framework was developed for HCC which included qualitative and quantitative performance indicators. This framework was then pressure tested and refined with 14 HCC MDT working groups within their respective hospitals, as well as with a cross-country panel of international HCC experts in a knowledge exchange session.

**Results:** A robust validated framework has been developed to analyse and assess HCC MDTs in a data-driven manner. The framework characterizes MDTs across three core elements: MDT process, MDT impact on decision-making, and MDT impact on patient outcomes. It includes 13 quantitative and 8 qualitative metrics, ranging from time to diagnosis and treatment to roles and responsibilities, that can be gathered in a reproducible manner.

**Conclusion:** The developed framework can be used by hospitals to self-assess their MDT maturity specifically when treating HCC and demonstrate the value of MDTs through impact measurement. As MDTs become critical for the patient care in HCC, this work is a first step to establish a standard of MDT value assessment and ways of working to support adoption and continuous improvement across hospitals.

# PO1-14-YI
# The impact of the onset of hepatocellular carcinoma on the natural history of cirrhosis

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**Background and aims:** Hepatocellular Carcinoma (HCC) is a leading cause of cancer related death and the large majority of HCC occur in the setting of chronic liver disease. Nevertheless, its impact on the development of decompensating events, such as ascites, hepatic encephalopathy (HE), portal hypertension related bleeding (PHB), and episodes of further decompensation (refractory ascites, spontaneous bacterial peritonitis [SBP] and hepatorenal syndrome [HRS]) in patients with cirrhosis has not yet been investigated. The aim of our study was to investigate the role of HCC in the development of decompensating events in patients with cirrhosis.

**Method:** 876 patients with cirrhosis were consecutively evaluated in the Outpatient clinic (CMP) of the Padua Teaching Hospital and followed up until death and/or liver transplantation (258 patients developed HCC, 618 patients without HCC during follow-up). Demographic, clinical, and laboratory data were collected, and patients were evaluated at least every 6 months between January 2000 and December 2022. The median follow-up time was 33 months. The primary outcome was the development of decompensating events after the diagnosis of HCC. HCC was considered as a time-varying covariate for the statistical analysis.

**Results:** Patients with HCC had a higher risk of developing a decompensating event (adjusted hazard ratio [aHR] = 3.07; p <0.001), such as ascites (aHR = 2.78; p <0.001), HE (aHR = 1.70; p = 0.002) and PHB (HR = 1.78; p = 0.021). As far as further decompensation, patients with HCC were at higher risk of developing refractory ascites (aHR 3.73; p <0.001), but not SBP (p = 0.50) and HRS (p = 0.09). As expected, patients with HCC had reduced overall survival (p <0.001). There was no difference in the incidence of decompensation based on the type of treatment. However, the response to the treatment itself could be associated with decompensation in these patients. Among patients with HCC, ALBI Grade was an accurate score to identify patients at higher risk of liver complications (p <0.001), and mortality (p = 0.029). The first treatment of HCC (surgical or locoregional therapy) reduced both the risk of decompensation (p = 0.005) and that of mortality (p <0.001).

**Conclusions:** The occurrence of HCC is associated with a high risk of decompensation and further decompensation in patients with cirrhosis.

# PO1-16
# Safety and efficacy of lenvatinib in very elderly patients with unresectable hepatocellular carcinoma

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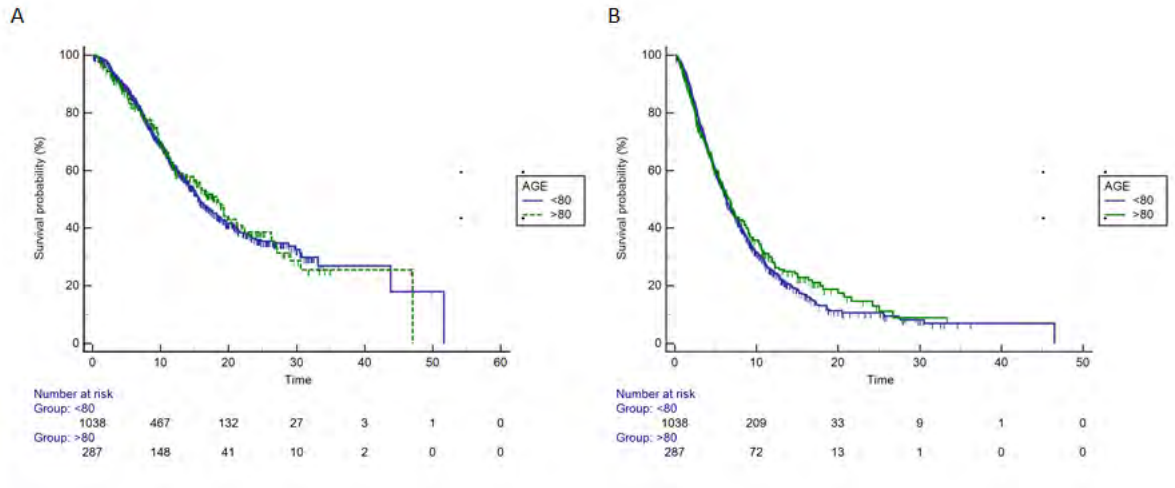
**Background and aims:** Data concerning the use of Lenvatinib in very elderly patients (≥80 years) are limited, although the incidence of hepatocellular carcinoma (HCC) in this patient population is constantly increasing. Here we present the results of efficacy and safety of Lenvatinib in a large cohort of very elderly patients (≥80 years) with unresectable HCC.

**Method:** The study was conducted on a cohort of 1325 patients from 46 centers of 4 Western and Eastern countries (Italy, Germany, Japan, and Republic of Korea) undergoing first line treatment with Lenvatinib between July 2010 and February 2022. Patients were stratified according to age in very elderly (≥80 years) versus not very elderly (<80 years).

**Results:** Median overall survival (OS) was 15.7 months for patients with <80 years and 18.4 for patients with ≥80 years, respectively (HR = 1.02, 95% CI 0.84-1.25, p = 0.8281). Median progression free survival (PFS) was 6.3 months for patients with <80 years and 6.5 months for patients with ≥80 years, respectively (HR = 1.07, 95% CI 0.91-1.25, p = 0.3954). No differences between the two study groups were found in terms of disease control rate (DCR) (80.8% vs. 78.8%; p = 0.44) and response rate (RR) (38.2% vs. 37.9%; p = 0.88). Patients with <80 years experienced significantly more Hand-Foot Skin Reaction (HFSR) grade ≥2 and decrease appetite grade ≥2; conversely patients with ≥80 years experienced significantly more fatigue grade ≥2. In the very elderly group parameters associated with prognosis were AFP, ALBI grade, BCLC and Child-Pugh score. BCLC stage was the only independent predictor of OS (HR = 1.59, 95% CI 1.11-2.29, p = 0.01115).

**Conclusion:** Our study highlights the same efficacy and safety of Lenvatinib between elderly and not elderly patients

**Figure:** Kaplan Meier for OS (A) and PFS (B) in patients treated with Lenvantinib



# PO1-17-YI
# ASAP score may predict HCC recurrence after complete radiological response to locoregional treatments

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**Background and aims:** Alpha fetoprotein (AFP) and prothrombin induced by vitamin K absence/antagonist II (PIVKA-II) are biomarkers for hepatocellular carcinoma (HCC), which have been extensively in the diagnosis of HCC, while their use in the prognosis prediction remains poorly assessed. Recently, a new algorithm (ASAP)-including age, gender, AFP and PIVKA-II-has been validated as an alternative to GALAD for prediction of HCC development with a cut-off of 0.52. The identification of predictors of HCC recurrence after curative treatment has always been relevant to patients' management, and now may have further utility with the arrival of adjuvant therapies.
The aim of our study was to evaluate the predictive role of AFP and PIVKA-II alone or combined in the ASAP score for HCC recurrence in patients who achieved a complete response (CR) after locoregional treatment.

**Method:** In this single-center, observational ongoing study, we have enrolled 156 consecutive patients with first diagnosis of HCC treated by ablation (MWTA) or chemoembolization (TACE). CR was evaluated by CT-scan 1 month after treatment, afterwards patients were evaluated every three months by CT-scan, clinical and biochemical features until recurrence, death or last follow-up. PIVKA-II and AFP levels were measured at the day of treatment by Fujirebio assays, Japan.

**Results:** 81 (52%) patients with HCC who achieved CR after the first treatment were included: median age 66 (40-87); 83% men, 57% HCV-positive, 91% Child-Pugh A, 85% BCLC 0/A, 53% MWTA. The day of treatment, the median AFP was 6.3 ng/ml (1.3-3, 537), median PIVKA-II was 112 (16-5, 090) mAU/ml, median ASAP score was 0.405 (-3.44-6.64; 47% with ASAP value >0.52). During follow-up, HCC recurred in 47 (58%) patients [median time to recurrence was 298 (41-1256) days after achieving CR]. PIVKA-II [HR 2.53 (95%CI 1.47-4.35), p = 0.001] and age [HR 1.03 (95%CI 1.00-1.07), p = 0.013] were the only independent predictors of overall HCC recurrence by a multivariable model for single variables only, while the ASAP score [HR 1.31 (95%CI 1.12-1.52), p <0.001] was the only independent predictor of recurrence in a multivariable model including only scores and algorithms. PIVKA-II [HR 2.14 (95%CI 1.09-4.18), p = 0.026] was the only independent predictor of early recurrence in the first multivariable model, while platelet to lymphocyte ratio [PLR; HR 1.02 (95%CI 1.00-1.04)] and ASAP score [HR 1.30 (95%CI 1.03-1.64)] were independent predictors of early recurrence according to the second model. Adopting the ASAP cut-off of 0.52, the algorithm independently predicted HCC recurrence [HR 3.54 (95%CI 1.86-9.75), p <0.001], but did not predict HCC early recurrence [HR 1.95 (95%CI 0.87-4.34), p = 0.103].

**Conclusion:** The ASAP algorithm may accurately predict HCC recurrence and early recurrence after complete radiological response, deserving further studies to refine the model.

# PO2-01
# A modified Charlson Comorbidity Index to improve management of patients with hepatocellular carcinoma: a step towards precision medicine

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**Background and aims:** Patients with hepatocellular carcinoma (HCC) frequently have comorbidities that limit the application of effective treatments and might increase mortality. However, comorbidities' assessment is not included in current staging and treatment systems. The Charlson comorbidity index (CCI) assigns a numeric score ranging from one to six to 17 diseases according to their effect on mortality. In patients with cirrhosis and HCC, these variables might be excluded from the CCI since they cannot be considered co-morbidities (modified CCI, mCCI). Aim of the study was to evaluate the performance of the CCI and mCCI for patients' characterization in HCC management.

**Method:** We used data from our retrospective monocentric study of patients with first diagnosis of HCC, where all the comorbidities included in the CCI have been reported. We evaluated the performance of CCI and of mCCI in dissecting our population and predicting overall survival. As secondary end point, the impact of CCI and mCCI on adherence to the Barcelona Clinic Liver Cancer (BCLC) staging and treatment allocation was evaluated.

**Results:** The study included 385 patients (289 males, 68 years median age, 78% Child Pugh (CPT) A, 65% viral etiology) with cirrhosis and HCC in different stages according to BCLC: 96 BCLC 0, 175 A, 64 B, 40 C and 10 D. According to the CCI, 94% of our patients were classified "high risk" (CCI score ≥5), 5.7% "intermediate risk" (CCI score 3-4) and 0.3% "low risk" (CCI score 0-2). The same cutoffs were applied to the mCCI: 22% "high risk", 50% "intermediate risk", and 28% "low risk". Patients' mCCI correlated with their CCI (Kendall's τ = 0.47; p <.0001). Overall, patients belonging to a "high" or "intermediate risk" classes according to mCCI had a shorter overall survival as compared to "low-risk" patients (median OS 37 vs 49 vs 69 months, respectively, p value = 0.02), corresponding to a 5-year survival rate of 27.3%, 42.3% and 53.5%, respectively. This observation was confirmed in BCLC stage 0/A (p value = 0.01), while no significant differences were observed in BCLC B and C. Moreover, "high risk" mCCI class, decreased liver function and AFP levels>200 ng/ml were independently associated to higher risk of mortality in BCLC 0/A patients [HR 1.36 (95%CI 1.02-1.81, p value = 0.03; HR 2.21 (95%CI 1.41-3.46), p value = 0.001; HR 1.97 (95%CI 1.11-3.42), p value = 0.02]. No differences were observed in the adherence to BCLC 2022 algorithm among the mCCI risk classes. Although, patients within the "high risk" mCCI class had lower access to radical treatments in BCLC B (p value = 0.002) as compared to intermediate and low risk patients.

**Conclusion:** The mCCI better stratified patients with HCC as compared to CCI and independently predicted mortality in early stages of disease. The implementation of a multiparametric therapeutic approach, including evaluation of comorbidities and frailty, to allocate treatment is warranted.

# PO2-03
# Treatment of hepatocellular carcinoma according to multiparametric therapeutic hierarchy approach: a prospective multicenter validation study

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**Background and aims:** Treatment strategy of hepatocellular carcinoma (HCC) is a complex decision-making process that considers several variables in clinical practice. A multiparametric therapeutic approach has been proposed (*Lancet Oncol* 2023): the feasibility of all treatments is evaluated systematically in hierarchical order, to match each patient with the optimal therapy. The aim of the study is to validate the reliability of the new multiparametric decisional framework (MPDF) and the impact of the different variables included in the framework on the final treatment decision in clinical practice.

**Method:** This is a prospective ongoing study with a first monocentric phase (A) and a second multicentric validation phase. Primary end points are: 1) adherence to MPDF for treatment allocation, 2) identification of the relative weight of variables involved in this MPDF for treatment decision, while secondary end points are overall survival and radiological response after treatment. The study includes all consecutive patients with HCC managed in each center from September 1st, 2023. The disease is staged according to Barcelona Clinic Liver Cancer (BCLC) classification, while patient's characterization is conducted by Charlson Comorbidity Index (CCI), Liver Frailty Index (LFI), Child-Pugh (CPT) and ALBI, MELD and skeletal muscle mass index by CT. Each patient is allocated to treatment according to MPDF, BCLC 2022 was the benchmark.

**Results:** As of November 1st, 2023, 48 patients were recruited into phase A of the study [median age 70 (52-87) years, 81% male, 37% HCV, 27% first treatment for HCC]. 13 (27%) patients were classified BCLC 0, 17 (35%) A, 6 (12.5%) B, 6 (12.5%) C and 6 (12.5%) D. The median CCI was 8 (4-13), the median LFI was 4.15 (2.99-4.81). 39 (81%) patients were in CPT class A, 7 (15%) in B and 2 (4%) in C, while 32 (67%) were classified ALBI grade 1 and 16 (33%) ALBI grade 2, and the median MELD score was 8.5 (6-22). Forty-four (92%) patients received treatment through a multidisciplinary team (MDT). According to the MPDF, 9 (19%) patients were allocated to liver transplant, 5 (10%) to laparoscopic surgical resection, 10 (21%) to microwave thermal ablation (MWTA), 7 (15%) to transarterial treatments (4 TACE and 3 TARE), 3 (6%) to other locoregional therapies (2 MWTA+TACE and 1 electrochemotherapy), 6 (13%) to systemic treatment and 5 (10%) to palliative care only. In 4 cases, the MDT decided for reassessment at 3 months. The reason why each therapy was excluded for each patient is shown in Figure. The treatment allocation was coherent to the BCLC 2022 in 69% BCLC 0, 82% BCLC A, 83% BCLC B, 67% BCLC C and 100% BCLC D.

**Conclusion:** Preliminary data of our ongoing prospective study suggest that personalized approach for HCC treatment by MPDF is feasible in clinical practice and might help ensuring a customized approach in a standardized framework. Updated data will be presented at the meeting.

## Figure:

| | |
|---|---|
| **Liver Transplant** | |
| Comorbidities | 11 (29.7%) |
| Extrahepatic extension of HCC | 6 (16.2%) |
| Beyond LT criteria | 5 (13.5%) |
| AFP>1000 | 1 (2.7%) |
| Unfeasible | 1 (2.7%) |
| Small benefit | 13 (35.1%) |
| **VLS Surgery** | |
| Frailty | 1 (3.1%) |
| Comorbidities | 14 (43.8%) |
| Extrahepatic extension of HCC | 6 (18.7%) |
| Multifocal HCC | 2 (6.3%) |
| CSPH | 4 (12.5%) |
| Liver disfunction | 2 (6.2%) |
| Limited liver volume remnant | 2 (6.3%) |
| Technical complexity | 1 (3.1%) |
| **Open Surgery** | |
| Frailty | 1 (3.1%) |
| Comorbidities | 13 (40.6%) |
| Extrahepatic extension of HCC | 6 (18.7%) |
| Multifocal HCC | 2 (6.3%) |
| CSPH | 3 (9.4%) |
| Liver disfunction | 5 (15.6%) |
| Limited liver volume remnant | 2 (6.3%) |
| **MWTA** | |
| Extrahepatic extension of HCC | 6 (30%) |
| > 3cm HCC | 4 (20%) |
| > 3 nodules of HCC | 2 (10%) |
| Critical location of HCC | 1 (5%) |
| Liver disfunction | 3 (15%) |
| Technical complexity | 2 (20%) |
| **VLS MWTA** | |
| ECOG PS > 2 | 1 (5.3%) |
| Extrahepatic extension of HCC | 6 (31.6%) |
| > 5cm HCC | 3 (15.8%) |
| > 3 nodules of HCC | 2 (10.5%) |
| Critical location of HCC | 2 (10.5%) |
| Liver disfunction | 3 (15.8%) |
| Technical complexity | 2 (10.5%) |
| **TACE** | |
| Extrahepatic extension of HCC | 6 (50%) |
| > 5 cm HCC | 1 (8.3%) |
| Multinodular and bilobar HCC | 2 (16.7%) |
| Liver disfunction | 3 (25%) |
| **TARE** | |
| Extrahepatic extension of HCC | 6 (54.5%) |
| Multinodular and bilobar HCC | 2 (18.2%) |
| Liver disfunction | 3 (27.3%) |
| **Systemic Therapy** | |
| ECOG PS>2 | 1 (25%) |
| Liver disfunction | 3 (75%) |

# PO2-04
# HCC incidence and risk stratification in patients with metabolic dysfunction-associated steatotic liver disease on long-term follow-up

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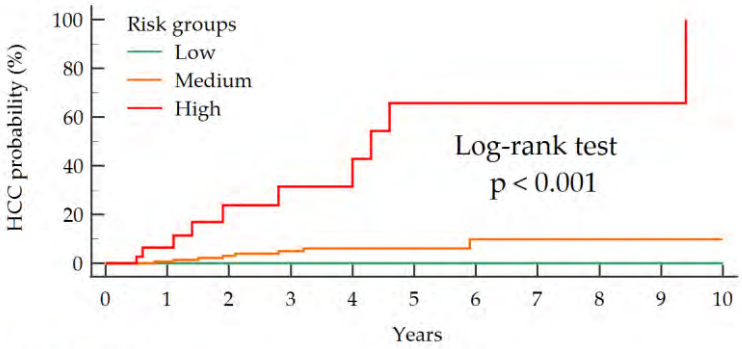
**Background and aims:** Metabolic dysfunction-associated steatotic liver disease (MASLD) is projected to become the leading cause of hepatocellular carcinoma (HCC) worldwide. Current surveillance strategy of patients at risk of HCC development is based on semestral US ± α-fetoprotein measurement. However, the identification of novel tools able to stratify the risk of HCC has the potential to reduce not only mortality but also to improve the cost-effectiveness of surveillance. Therefore, we aimed to investigate the performance of non-invasive tests (NITs) in comparison or in combination to protein induced by vitamin K absence or antagonist II (PIVKA-II) for the stratification of the risk of HCC development in patients with MASLD on long-term follow-up (FU).

**Method:** We retrospectively enrolled 366 patients with MASLD and significant liver fibrosis (LSM ≥8 kPa or F≥2 at liver biopsy). Median age was 58 (IQR 49-66) years, 198 (54.1%) patients were males, and 168 (45.9%) patients had a diagnosis of type-2 diabetes mellitus. Compensated advanced chronic liver disease (cACLD) (LSM ≥10 kPa or F≥3 at liver biopsy) was diagnosed in 279 (76.2%) patients. All patients had at least 6 months of FU with regular US surveillance. The following NITs were calculated at baseline: AST to platelet ratio index (APRI), Fibrosis-4 score (FIB-4), and NAFLD fibrosis score (NFS). Serum PIVKA-II was measured by CLEIA on Lumipulse® G600 II platform (Fujirebio Inc., Tokyo, Japan). The accuracy for HCC prediction was evaluated by Harrell's C statistic (*C*).

**Results:** During a median FU of 2.9 (IQR 1.0-4.3) years, 19 out of 366 (5.2%) patients developed HCC (incidence rate: 1.49 per 100 person/years). Notably, all the 19 HCC occurred in patients with cACLD (n = 279) (incidence rate: 2.06 per 100 person/years). At HCC diagnosis, BCLC stage was 0 in 4 (21.1%) patients, A in 10 (52.6%), B in 3 (15.8%), C in 1 (5.3%); BCLC stage was unknown in 1 patient.
In the whole cohort of 366 patients, NFS showed the best performance for HCC prediction (*C* = 0.81), followed by FIB-4 (*C* = 0.78), and by APRI (*C* = 0.71); the accuracy of serum PIVKA-II was *C* = 0.76. In patients with cACLD (n = 279), the predictive performance of NITs was *C* = 0.77 for NFS, *C* = 0.75 for FIB-4, and *C* = 0.68 for APRI, while PIVKA-II showed an accuracy of *C* = 0.74. Remarkably, in patients with cACLD the combination of NFS + PIVKA-II significantly improved the accuracy for HCC prediction (*C* = 0.84), allowing to stratify patients into 3 risk categories with different HCC incidence: low-risk (0/52; 0%), medium-risk (9/190; 4.7%), and high-risk (10/37; 27.0%) (Log-rank test: p <0.001; Figure).

**Conclusion:** In patients with MASLD and significant fibrosis, NFS showed appropriate performance for the prediction of HCC development. However, in patients with more advanced liver disease, the measurement of serum PIVKA-II may be a useful diagnostic complement to improve HCC prediction and risk stratification.

**Figure:**




Number at risk

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group: Low | | | | | | | | | | |
| 52 | 49 | 40 | 34 | 20 | 12 | 10 | 9 | 8 | 6 | 5 |
| Group: Medium | | | | | | | | | | |
| 190 | 136 | 109 | 88 | 47 | 29 | 22 | 16 | 12 | 11 | 8 |
| Group: High | | | | | | | | | | |
| 37 | 19 | 11 | 8 | 5 | 2 | 2 | 1 | 1 | 1 | 0 |

# PO2-05-YI
# Characteristics and outcomes of immunotherapy-related liver injury in patients with hepatocellular carcinoma compared to patients with advanced solid tumours

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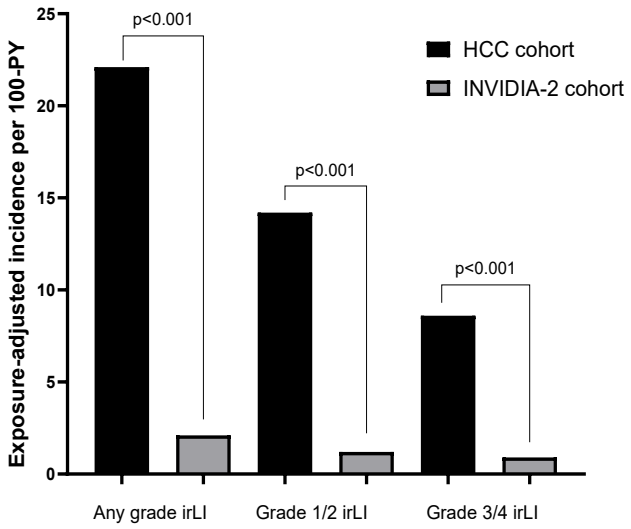
**Background and aims:** Immune-related liver injury (irLI) is commonly observed in patients with cancer treated with immune checkpoint inhibitors (ICIs). However, it is unknown if irLI is more frequent or if it negatively affects outcomes in patients with hepatocellular carcinoma (HCC). In this comparative study, we aimed to compare incidence, clinical characteristics and outcomes of irLI between patients receiving ICIs for HCC versus other solid tumour indications.

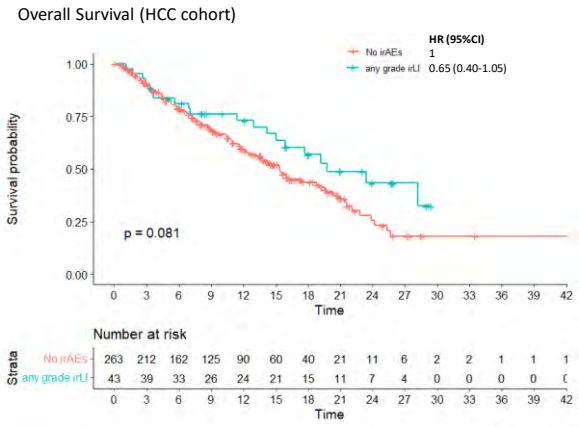
**Method:** Two separate cohorts were included:375 patients with advanced/unresectable HCC, Child-Pugh A class treated with first-line Atezolizumab+Bevacizumab from AB-real study and a non-HCC cohort, including 459 patients treated with first-line ICI therapy from INVIDIa-2 multicentre study. IrLI was defined as treatment-related increase of transaminases levels after exclusion of alternative aetiologies of liver injury. Incidence of irLI was adjusted for the duration of treatment exposure.

**Results:** In HCC patients, incidence of any-grade irLI was 11.4% over a median treatment exposure of 4.4 months (95%CI 3.7-5.2), compared to 2.6% in INVIDIa-2 cohort over a median treatment exposure of 12.4 months (95%CI 11.1-14.0). Exposure-adjusted incidence of any-grade irLI was 22.1 per 100-Patient-years (PY) in HCC patients and 2.1 per 100-PY in non-HCC patients (p <0.001), with median time to irLI of 1.4 in HCC and 4.7 months in non-HCC patients, respectively. Among patients who developed irLI, systemic corticosteroids were administered in 16.3% of HCC and in 75.0% of non-HCC patients (p <0.001) and irLI resolution was observed in 72.1% and 58.3%, respectively (p = 0.362). In HCC patients, rates of hepatic decompensation and treatment discontinuation due to irLI were 7%. In both cohorts, no fatal irLI events occurred. Development of grade 1-2 irLI was associated with improved overall survival in HCC patients only (HR 0.53, 95%CI 0.29-0.96).

**Conclusion:** Despite higher incidence and earlier onset in patients with HCC, IrLI is characterised by high rates of remission, low requirement for corticosteroid therapy and low risk of decompensation compared to other solid tumours. Hepatotoxicity leads to discontinuation in 7% of patients with HCC and does not negatively affect oncological outcomes.

**Figure:**


Exposure-adjusted incidence per 100-PY
25
20
15
10
5
0
p<0.001
p<0.001
p<0.001
HCC cohort
INVIDIA-2 cohort
Any grade irLI
Grade 1/2 irLI
Grade 3/4 irLI


Overall Survival (HCC cohort)
HR (95%CI)
No irAEs 1
any grade irLI 0.65 (0.40-1.05)
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.081
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42
Time
Number at risk
Strata
No irAEs 263 212 162 125 90 60 40 21 11 6 2 2 1 1 1
any grade irLI 43 39 33 26 24 21 15 11 7 4 0 0 0 0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42
Time

## PO2-06-YI
# Exploring the role of aMAP and Toronto score: beyond diagnosis to prognosis

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**Background and aims:** Hepatocellular carcinoma (HCC) is the leading cause of primary liver cancer and the third cause of tumor death. The early identification of HCC is fundamental to providing patients with the most curative treatment possible. aMAP score and Toronto score have been widely validated for defining the first occurrence of HCC. The early recurrence of HCC after treatment is a negative prognostic factor and it is related to both treatment and patient features. To date, no validated scores to predict the risk of early HCC recurrence, after therapies, have been validated. Aim: to evaluate the accuracy of aMAP and Toronto scores to predict the early recurrence of HCC after locoregional treatment.

**Method:** We consecutively enrolled 126 patients with HCC from 2014 to 2020. Patient staging and treatment choice by using the Barcelona Clinic Liver Cancer (BCLC) system. The early recurrence of HCC was defined by the recurrence of HCC within the 2 years after the first treatment. We used aMAP and Toronto score as linear variables and with the cut-off proposed in literature for the first HCC occurrence (>60 and>240 respectively).

**Results:** The mean age was 69 ± 11 years, 77% were males. The primary etiologies of cirrhosis were viral in 55%, alcohol-related in 22%, metabolic in 19%, and iron overload-related in 4%. Even in patients in whom the etiologic diagnosis of cirrhosis was not metabolic, diabetes and obesity were present in 59%. At the diagnosis of HCC 41% had more than one lesion, 12% underwent surgical resection, 77% locoregional treatment, 3% systemic therapy, and 8% best supportive care. We focused our analysis on only 96 patients with locoregional treatment. Among them, 60% had an early HCC recurrence. For the prediction of early recurrence the accuracy of aMAP and Toronto scores, considered both as linear variables (AUROC 0.63 for both aMAP and Toronto score) and with the cut-offs proposed in the literature (AUROC 0.52 and 0.56), were low. To optimize sensibility and sensitivity of the scores an aMAP score greater than 74 and a Toronto score greater than 250 should be used (sensibility and specificity 54% and 75% for aMAP score and 65% and 63%, for Toronto score). In patients with metabolic comorbidities, the accuracy of both aMAP and Toronto score for early recurrence was further reduced (AUROC of aMAP 0.40 vs 0.62 in patients with and without metabolic comorbidities, respectively; AUROC of Toronto 0.50 vs 0.76, respectively).

**Conclusion:** both aMAP and Toronto scores did not show good accuracy in predicting early HCC recurrence after locoregional therapies. The presence of metabolic comorbidities, such as diabetes and obesity, negatively affected both scores. Due to the significant implications of early recurrence in the management of HCC patients, there is a need to develop and validate specific scores for the assessment of early recurrence, taking into account the presence of diabetes and obesity.

# PO2-08-YI
# Aetiology of hepatocellular carcinoma and response to immunotherapy: is the the problem inherent in the classification of non-viral disease?

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**Background and aims:** Preclinical studies and certain post-hoc analyses of randomized clinical trials hint at a possible impaired efficacy of immune checkpoint inhibitors in patients with non-viral hepatocellular carcinoma (HCC) in general and metabolic dysfunction-associated steatotic liver disease (MASLD) in specific. The heterogeneity of non-viral aetiologies, the possibility of multiple concurrent aetiologies may justify seemingly discordant data. We aimed to explore and compared the objective response rate [ORR], disease control rate [DCR], progression-free survival [PFS], and overall survival [OS] of HCC patients treated with atezolizumab/bevacizumab (AB), stratified according different criteria for non-viral etiologies and MASLD.

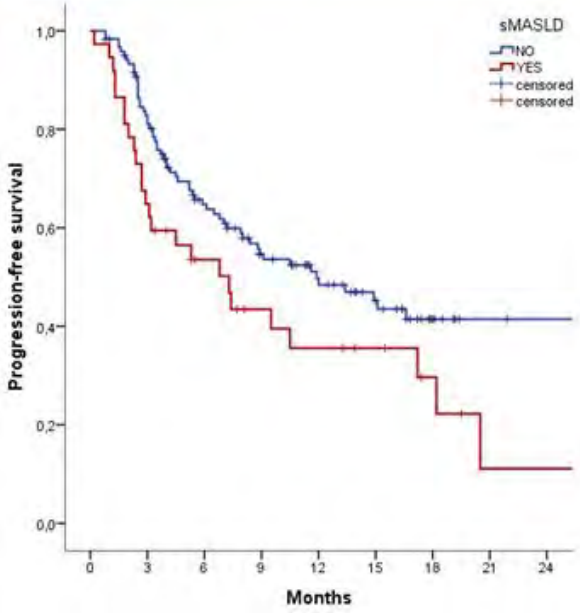
**Method:** The ARTE database (March 2022-November 2023) prospectively enrolled patients treated with AB in a real-life setting. Three different classifications of HCC aetiologies were explored: 1) viral vs non-viral; 2) MASLD (either single-etiology or combined with other etiologies, for instance HCV) vs non-MASLD; 3) single-etiology MASLD (sMASLD) vs non-sMASLD. ORR, DCR, PFS and OS comparisons were performed using both univariable analyses and multivariable models including other predictors of outcome.

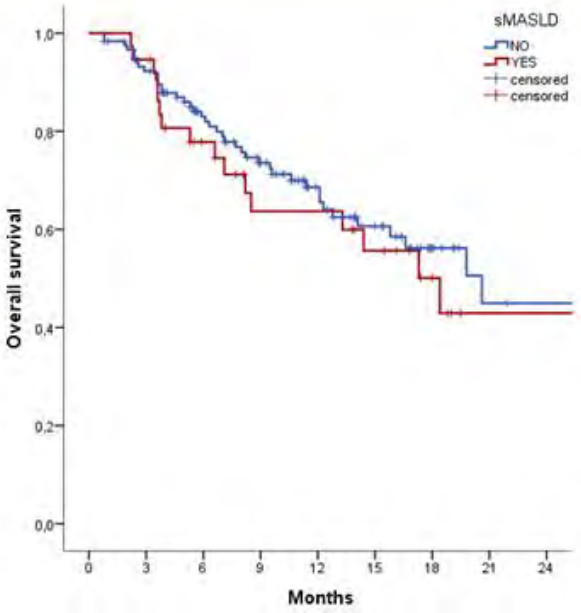
**Results:** Data of 157 patients from 12 centres were collected. The ORR, DCR, median PFS and OS in the whole study population were: 19.7%, 62.4%, 19.8 (95% CI 15.8-23.8) and 10.5 months (6.3-14.7), respectively . Stratification according to the viral vs non-viral etiologies did not capture differences in the main outcome measures (Figure). Conversely, patients with sMASLD had shorter PFS and a trend toward a lower DCR compared to controls, but without differences in OS.

**Conclusion:** Viral and non-viral aetiologies had no significant differences in their response to AB. Patients with sMASLD had a shorter PFS than controls, but this difference did not translate into an impaired survival. These data confirm that aetiology alone should not preclude patients from receiving immune-oncology drugs, based on current evidence. A longer follow-up might be useful to understand the possible confounding effects from second-line therapies on the interpretation of OS.

**Figure:**

| | | Non-viral vs viral | MASLD (yes vs no) | sMASLD (yes vs no) |
|---|---|---|---|---|
| ORR | % | 18.3 vs 20.9 (p=0.84) | 19.6 vs 19.8 (p=1.00) | 13.5 vs 21.7 (p=0.35) |
| | *OR* | 1.16 (0.48-2.78, p=0.87) | 1.07 (0.43-2.67, p=0.99) | 0.67 (0.22-2.00, p=0.38) |
| DCR | % | 59.3 vs 66.2 (p=0.41) | 51.8 vs 68.3 (p=0.06) | 51.4 vs 65.8 (p=0.12) |
| | *OR* | 0.71 (0.34-0.49, p=0.29) | 0.55 (0.27-1.15, p=0.11) | 0.57 (0.26-1.39, p=0.18) |
| PFS | *months* | 8.8 vs 12.0( p=0.77) | 7.3 vs 11.9 (p=0.05) | 7.3 vs 11.9 (p=0.02) |
| | *HR* | 1.05 (0.64-1.72, p=0.81) | 1.26 (0.80-2.00, p=0.31) | 1.88 (1.13-3.12, p=0.01) |
| OS | *months* | 18.4 vs 19.8 (p=0.79) | 18.4 vs 19.8 (p=0.47) | 18.4 vs 20.6 (p=0.28) |
| | *HR* | 1.16 (0.63-2.15, p=0.63) | 0.99 (0.56-1.78, p=0.95) | 1.37 (0.74-2.53, p=0.32) |



# PO2-10
# Impact of body mass index on the prognosis of unresectable HCC patients receiving first line Lenvatinib or Atezolizumab plus Bevacizumab

Noemi Cornara[1 2], Bernardo Stefanini[3], Margherita Rimini[4], Toshifumi Tada[5], Goki Suda[6], Shimose Shigeo[7], Masatoshi Kudo[8], Fabian Finkelmeier[9], Changhoon Yoo[10], José Presa[11], Elisabeth Amadeo[1], Massimo Iavarone[12], Fabio Marra[13], Francesco Foschio[14], Emiliano Tamburini[14], Federico Rossari[1], Francesco Vitiello[1], Sara Lonardi[15], Marianna Siletta[16], Mara Persano[17], Silvia Camera[17], Stefano Cascinu[2], Andrea Casadei-Gardini[4], Fabio Piscaglia[18]

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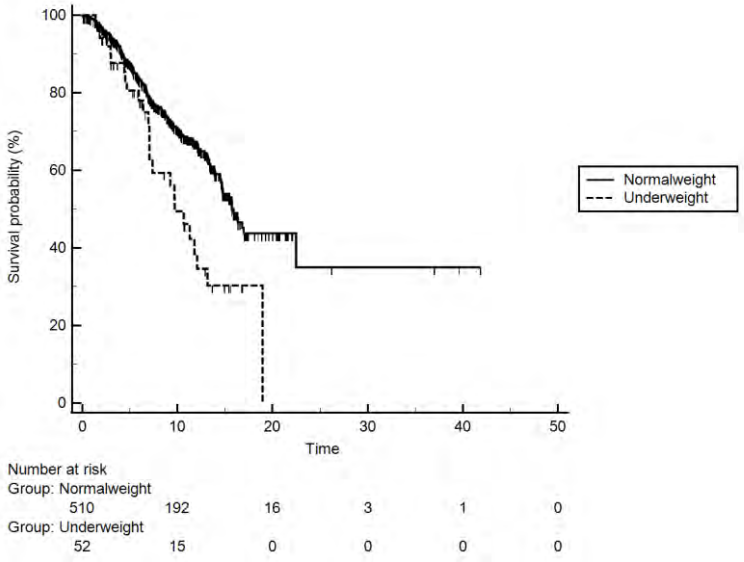
**Background and aims:** Overweight is a negative prognostic factor in the general population in the long term. However, the role of Body mass index (BMI) in the short-mid term in advanced tumors is unclear. The present analysis investigates the role of BMI weight classes in a large sample of patients affected by HCC and receiving Atezolizumab plus Bevacizumab or Lenvatinib as first line treatment.

**Method:** The cohort included consecutive patients affected by BCLC-c and BCLC-B HCC patients from a multicenter international study group who received Atezolizumab plus Bevacizumab or Lenvatinib as first line therapy. Population was stratified according to the BMI in under-, over- and normal-weight according to the conventional thresholds. The primary objective of the study was to evaluate the prognostic and predictive impact of BMI in patients affected by advanced or intermediate HCC. Survival curves were estimated using the product-limit method of Kaplan-Meier. The role of stratification factors was analyzed with log-rank tests.

**Results:** 1292 consecutive patients with HCC were analyzed. 466 (36%) patients were treated with Lenvatinib and 826 (64%) patients were treated with Atezolizumab plus Bevacizumab. In the Atezolizumab plus Bevacizumab arm, 510 (62%) patients were normal-weight, 52 (6%) underweight and 264 (32%) overweight. At the univariate analysis for OS, underweight patients had significantly shorter OS compared to normal-weight patients, whereas no differences were found between normal-weight versus overweight. Multivariate analysis confirmed that underweight patients had significantly shorter OS compared to normal-weight patients (HR 1.7; 95% CI, 1.0-2.8; p = 0.0323). In the Lenvatinib arm, 26 patients (5.6%) were categorized as underweight, 256 (54.9%) as normal-weight, and 184 (39.5%) as overweight. At the univariate analysis for OS, no significant differences were found between normal-weight versus underweight and between normal-weight versus overweight, which was confirmed at multivariate analysis.

**Conclusion:** Our analysis highlighted a prognostic role of BMI in a cohort of patients with advanced HCC who received Atezolizumab plus Bevacizumab, while no prognostic role for low BMI was apparent in patients who received Lenvatinib.

**Figure:**


100
80
60
40
20
0
Survival probability (%)
0
10
20
30
40
50
Time
Normalweight
Underweight
Number at risk
Group: Normalweight
510 192 16 3 1 0
Group: Underweight
52 15 0 0 0 0

## PO2-11-YI

# The presence of hepatocellular carcinoma (HCC) does not influence the rebalanced hemostasis in patients with cirrhosis

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**Background and aims:** Conventional coagulation tests (CCTs) are unreliable predictors of the hemostatic balance in patients with advanced liver disease. Thromboelastography (TEG) provides a global assessment of coagulation, evaluating clotting factors (R-time), fibrinogen (K, alpha angle), platelet function (maximum amplitude-MA), and fibrinolysis (Ly30). Empirically, the presence of a malignancy is expected to generate a procoagulant state. The current study evaluated whether the presence of hepatocellular carcinoma (HCC) is associated with changes in the coagulation profile in patients with cirrhosis.

**Method:** A proof of concept study was designed, including consecutive patients with liver cirrhosis and abnormal CCTs (at least one of International Normalized Ratio-INR >2, platelet count <50.000/μL, fibrinogen <200 mg/dL), subsequently analyzed using native TEG and compared with a matched cohort of patients with a similarly staged cirrhosis and HCC.

**Results:** A series of 106 consecutive patients were retrospectively analyzed, of which n = 22 (20.75%) had HCC. On the whole group analysis, there were no statistically significant differences between patients with or without HCC regarding either CCTs or TEG-based variables. A 1:1 propensity-matched analysis included patients staged Child-Pugh A and B, taking into account the MELD-Na score and CCTs, which included 44 patients. In the setting of a similar conventional coagulation profile, patients with HCC had no significant differences regarding TEG-based variables: R-time 10.62 ± 5.99 vs. 10.92 ± 3.57 min (p = 0.84), K-time 5.69 ± 4.38 vs. 6.08 ± 3.30 min (p = 0.74), alpha angle 42.11 ± 16.66 vs. 37.28 ± 14.31 (p = 0.32), MA 52.94 ± 12.60 vs. 49.05 ± 16.06 (p = 0.38), and Ly30 1.61 ± 1.99 vs. 1.48 ± 2.81 (p = 0.86), for patients without and with HCC, respectively.

**Conclusion:** The presence of HCC in the setting of advanced liver disease does not appear to significantly impact the rebalanced hemostasis, as assessed by TEG.

# PO2-12
# The synergic effect of metformin with atezolizumab/bevacizumab in MASLD HCC patients: a retrospective study from ARTE multicentric Italian dataset

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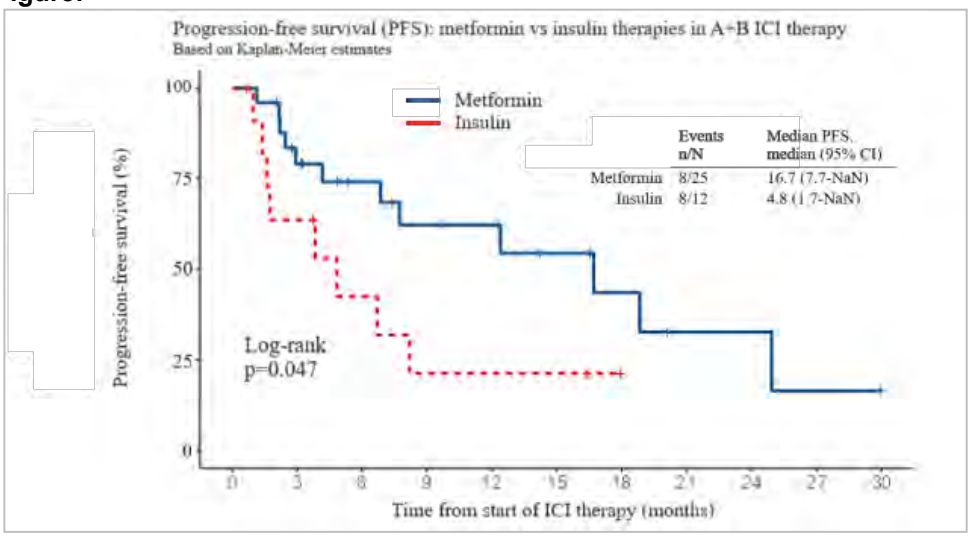
**Background and aims:** The standard treatment of advanced HCC is immune checkpoint inhibitor (ICI) therapy. Metabolic dysfunction-associated steatotic liver disease (MASLD) appears to adversely affect the efficacy of ICI. Recently, the antidiabetic drug metformin has garnered attention for its possible antitumor and immunomodulatory properties, such as reduction of proinflammatory cytokines and CD8+ T cells activation during immunotherapy. The aim of our study was to investigate the role of metformin in patients treated with atezolizumab/bevacizumab (A+B).

**Method:** 159 HCC patients (82% males, mean age 64.5) treated with A+B were enrolled from ARTE dataset. Clinical and radiological factors associated with patients' response to therapy were used to stratify objective response rate (ORR), overall survival (OS) and progression free survival (PFS) by Kaplan- Meier methodology, followed by Log-rank test in multivariate analysis.

**Results:** 53.3% patients had MASLD, with 31.9% being diabetic. No differences in OS, PFS and ORR were documented among the different etiologies. Considering 31 ORR patients, no differences between the two groups were underlined regarding sex, age, liver disease etiology. In the multistep multivariate model, diabetes was the only condition remained independently associate to ORR (OR 3.0, 95%CI 1.0-8.3; p = 0.030). When diabetic patients were stratified based on antidiabetic treatments, those on metformin had a 12 months survival of 62% [44%-88%, 95% CI] vs insulin treatment with 21% [6%-72%, 95% CI], p <0.05.

**Conclusion:** Despite any clear difference in terms of ORR, OS and PFS between different etiologies, diabetic patients treated with metformin exhibited a better ORR and PFS, suggesting a potential immunological combination role of metformin with A+B treatment.

**Figure:**


Progression-free survival (PFS): metformin vs insulin therapies in A+B ICI therapy
Based on Kaplan-Meier estimates
Metformin
Insulin
Events n/N
Median PFS, median (95% CI)
Metformin 8/25 16.7 (7.7-NaN)
Insulin 8/12 4.8 (1.7-NaN)
Log-rank p=0.047
Progression-free survival (%)
100
75
50
25
0
0
3
6
9
12
15
18
21
24
27
30
Time from start of ICI therapy (months)

# PO2-13-YI
# Modeling cancer cells and tumor vasculature dynamics by serum biomarkers in HCC patients with different response to TKIs, TACE and TARE suggests a synergistic effect of systemic and endovascular treatments

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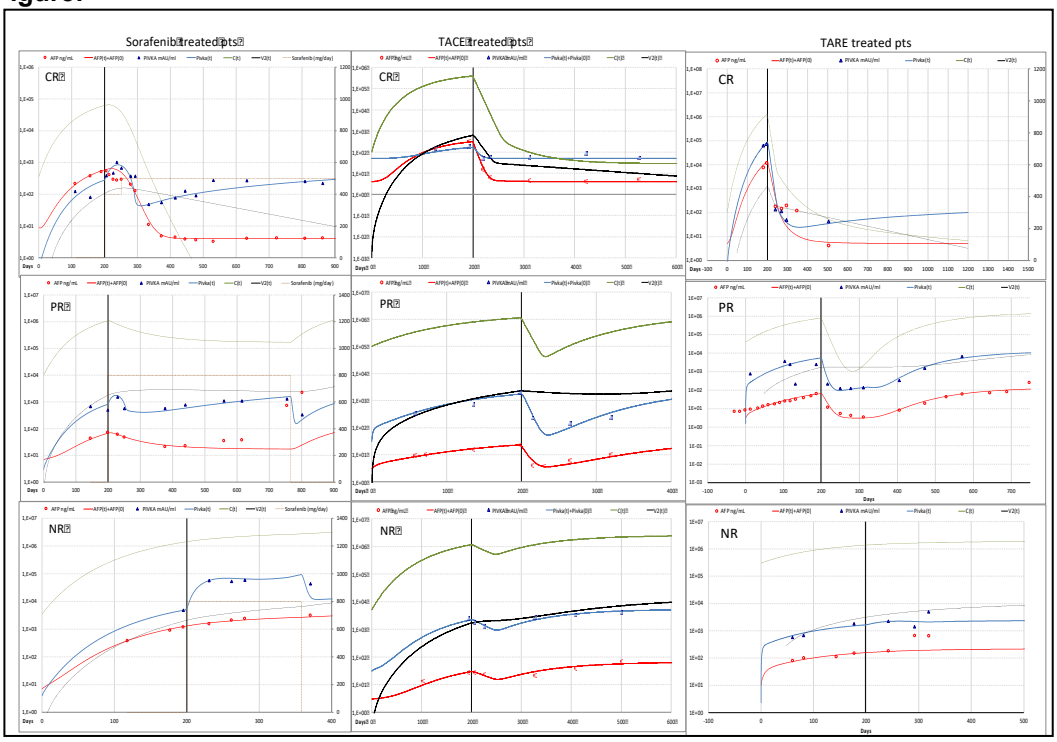
**Background and aims:** Tyrosine kinase inhibitors (TKI), trans-arterial chemo-embolization (TACE) and trans-arterial radio-embolization (TARE) are used in patients (pts) with unresectable hepatocellular carcinoma (HCC).Previously, we proposed a physic-mathematical model to study cancer cells and tumor vasculature dynamics (doi:10.3390/cancers13092064) by serum α-fetoprotein (AFP) and protein induced by vitamin K absence-II (PIVKA-II) kinetics integrated with digital imaging. We here quantified by the model TKIs, TACE and TARE therapeutic effects in patients with complete (CR), partial (PR) and no (NR) response.

**Method:** Ten pts (2-F and 8-M; median age: 65y; stage: 1-BCLC-B and 9-BCLC-C) received TKIs (1 regorafenib, 9 sorafenib), eight (5-F and 3-M; 77y; all BCLC-B stage) TACE (doxorubicin+DC-beads) and seven (1-F and 6-M; 70y; all BCLC-B stage) TARE (Yttrium-90). AFP and PIVKA-II were tested by commercial assays (Abbott, Fujirebio). HCC volume/densitometry were measured by CT scans (GE Advantage Workstation 4.6).

**Results:** Median HCC volume was greater in TKI vs TARE and TACE pts (42.1 vs 29.9 and 9.7 cm 3 ; p = 0.065).Changes of AFP and PIVKA-II serum values in 10 TKI (4 CR, 4 PR, 2 NR), 7 TARE (3 CR, 3 PR, 1 NR) and 8 TACE (2 CR, 5 PR, 1 NR) pts were fitted into the model. Anti-angiogenesis and anti-proliferative effectivenesswere higher in CR vs non-CR (30.0 vs 10.0, p = 0.023 and 10.0 vs 1.0, p = 0.043), and in TKI vs TARE and TACE (median 40.0 vs 18.0 vs 3.0, p = 0.015 and 4.0 vs 1.0 vs 0.5, p = 0.013). TACE and TARE, however, reached maximal therapeutic effect earlier (andlt;1.0 vs 4.6-99.9 days), due to the higher drug uptake coefficient (median: 0.0005 and 0.00035 vs 0.0000075, pandlt;0.001). Accordingly, AFP and PIVKA-II reduction occurred immediately after TACE and TARE, with AFP half-life comparable to its natural decay (0.10-0.16 day -1 ). By contrast, AFP decline after TKI was delayed, and an early spike of PIVKA-II levels occurred in most responders, suggesting that PIVKA-II production rate by neoplastic cells increases transiently with slow ischemia onset.

**Conclusion:** The anti-angiogenetic and anti-proliferative effectiveness of TKI, TARE and TACE, computed bymodeling AFP and PIVKA-II decline, correlates with the efficacy of the treatments. Their different modes of action are captured by the model, suggesting a synergic effects of TKIs and endovascular treatments. Future trials could assess whether model tailored schedules increase therapeutic efficacy.

**Figure:**


Sorafenib treated pts
TACE treated pts
TARE treated pts
CR
CR
CR
PR
PR
PR
NR
NR
NR

# PO2-14-YI
# Non-alcoholic fatty liver disease following management of ovarian cancer

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**Background and aims:** Chemotherapy induced liver injury is one of the common causes of mortality in cancer patient. One of the mechanisms of development of fatty liver is hepatic steatosis induced by chemotherapeutic agents. Here in this study we are evaluating the development of fatty liver during the therapy for ovarian cancer.

**Method:** A prospective study was conducted on cases of ovarian cancer with normal liver function test, which developed fatty liver after getting chemotherapy.

**Results:** We studied 200 cases of ovarian cancer, out of which 31 (15%) cases developed fatty liver secondary to therapy for ovarian cancer. The mean value of age that developed fatty liver is 49.2 years. Among various clinical parameters, only weight and body surface area (BSA) did show a statistically significant correlation (p = 0.05) with the development of fatty liver. The patients who had PFI more than 15 months also showed the development of FL (p = 0.03) (table 1).

**Conclusion:** Development of fatty liver following chemotherapy follows the common mechanism, but the process is fast. This may be due to an altered metabolic process. Here also weight and BSA are associated with the development of fatty liver. Following chemotherapy, progression-free interval also has shown a significant correlation with the development of fatty liver.

**Figure: Table 1: A statistical correlation between the developments of fatty liver with different clinical parameters (Point-Biserial Correlation)**

| | | FL (N = 31) | | |
|---|---|---|---|---|
| | **Mean values** | **N** | **Correlation coefficient** | **P value** |
| Age | 49.2 ± 7.95 | 31 | 0.04083397 | 0.5089 |
| Height | 151 ± 8.2 | 30 | -0.02402475 | 0.7565 |
| Weight | 59.37 ± 13.93 | 31 | 0.1501248 | 0.0521 |
| BSA | 1.56 ± 0.2 | 30 | 0.1533173 | 0.05292 |
| CA125 | 68848.6 ± 110251.5 | 18 | 0.08643838 | 0.2786 |
| HE4 | 452.28 ± 564.22 | 11 | 0.02149196 | 0.812 |
| CA19.9 | 61.12 ± 129.62 | 21 | -0.01088773 | 0.8955 |
| CEA | 1.8 ± 1.13 | 21 | -0.05292008 | 0.5159 |
| GFR | 88.4 ± 24.9 | 18 | -0.04770862 | 0.6608 |
| Tumor Size | 11.77 ± 6.6 | 24 | -0.03569661 | 0.652 |
| FCB | 18.36 ± 7.32 | 19 | -0.1035075 | 0.2586 |
| LCB | 26.5 ± 11.2 | 17 | -0.01796504 | 0.8515 |
| Menarche | 13.6 ± 1.1 | 10 | 0.05972043 | 0.6392 |
| Menopause | 45.7 ± 5 | 22 | 0.04035766 | 0.6227 |
| PFI | 15.4 ± 12.2 | 5 | 0.3778583 | 0.03016 |

# PO2-15-YI
# The impact of transjugular intrahepatic portosystemic shunt on hepatocellular carcinoma : an analysis of a prospective cohort of liver transplant candidates

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**Background and aims:** Transjugular Intrahepatic Portosystemic Shunt (TIPS) is used to mitigate the side effects of portal hypertension. Its impact on hepatocellular carcinoma (HCC) remains unclear. We aimed to measure its impact on HCC growth and carcinogenesis and evaluate its effect on patient's survival.

**Method:** We conducted an analysis of the prospective Scientific Registry of Transplant Recipients (SRTR) and included 43'734 liver transplant candidates with HCC. Using propensity score matching, 7'404 patients with and without TIPS were paired at a 3:1 ratio. We assessed wait-list changes in total tumor volume, number of HCC, and alpha-fetoprotein. We examined survivals from listing and from transplantation, and the incidence of post-transplant HCC recurrence.

**Results:** Before matching, patients with TIPS demonstrated a worse liver function and less advanced HCCs compared to patients without TIPS. After matching, TIPS was associated with a decrease in the number of HCC nodules (-0.24 vs 0.11, p = 0.008) over a median waiting time of 225 days (IQR 94; 441) and a better overall survival from listing (93.0% vs 89.1% at one year, p = 0.0003). TIPS was not associated with waitlist increase in tumor volume (0.26 vs -0.07 cm3/month, p = 0.26). Both post-transplant survival (91.8% vs 91.7% at one year, p = 0.25 and HCC recurrence (5.2% vs 5.4% at 5 years, p = 0.73) were similar between groups with a median follow-up of 5.6 years (IQR 2.7; 9.6).

**Conclusion:** TIPS is associated with an improved waitlist survival and a decrease in the number of HCC, potentially thanks to a better efficacy of HCC treatment. TIPS has no measurable impact on HCC growth.

# PO2-16-YI
# Profile of immune-related adverse events and treatment outcomes in first-line immunotherapy for hepatocellular carcinoma

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**Background and aims:** The combination of Atezolizumab+Bevacizumab (AB) and Tremelimumab+Durvalumab (TD) are the first-line systemic treatments for hepatocellular carcinoma (HCC). Additionally, Durvalumab (D) and Lenvatinib demonstrated to be non-inferior to sorafenib. The aim of this study is to describe the profile of immune-related adverse events (irAE) that condition temporally treatment interruption (interruption) and/or discontinuation, as well as outcome after AB, TD or D.

**Method:** Patients (p) treated with first-line immunotherapy from two medical centres in Spain were assessed retrospectively. Demographic characteristics were recorded, as well as the number and type of irAEs that conditioned interruption or discontinuation, date of appearance of irAEs, date and reason for interruption/discontinuation of AB, TD or D and type of 2nd line treatments received. The irAE was defined as all those AE ≥grade II in which the relationship with AB, TD or D cannot be excluded.

**Results:** Of the 109 p included in the study, 83 p were treated with AB, 23 p with TD and 3 p with D. Among them, 52.3% were BCLC-C, 84.4% were Child-Pugh A and the remaining patients did not have cirrhosis. The median follow-up was 8.5 months [range 4.6-16.6], during this period 55 (50%) patients interrupted treatment due to irAE and 32 (29.4%) died, 2p (1.83%) for grade 5 irAE. The median time until the first irAE that led treatment interruption was 64 days [21 -192] and 12.8% of patients developed it after the first dose of immunotherapy. The most frequent AEs were proteinuria (7p), diarrhea/colitis (5p), arterial hypertension (4p), myocarditis (4p) and encephalitis (4p).
Out of the total number of patients, 53.2% discontinued treatment: 22p due to due to symptomatic progression, 16p radiological progression, 15p AEs (not all irAEs), 1p by patient's decision and 4p for other reasons (3 for a second neoplasia and 1 for an unrelated death). Of those who discontinued treatments such as AB, TD, or D, 29.3% (17p) went on to receive second-line treatment, with 53% (9p) participating in second-line clinical trials.

**Conclusion:** Although 53.2% of patients discontinued the 1st line immunotherapy schemes, only in 13.8% of them was due to adverse events. The median time to the first adverse event that required treatment interruption was 64 days, and in 12.8% of the patients, this adverse event occurred after the first dose of immunotherapy. This data emphasizes the importance of establishing hospital networks for early detection and ensuring timely access to experienced specialists for the management of immune-related adverse events.

# PO2-17
# Knowledge of ultrasound LI-RADS for hepatocellular carcinoma surveillance: a comparison between two centres

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**Background and aims:**
Ultrasound Liver Imaging reporting and Data System (US LI-RADS) is a standardised system for performing, interpreting and reporting US scans for hepatocellular carcinoma (HCC) surveillance. Our aim was to compare US LI-RADS knowledge between two UK centres.

**Method:** Waiving ethical approval, practitioners performing HCC surveillance US at two centres (hospital 1: established US LI-RADS service versus hospital 2: US LI-RADS not implemented) were invited to complete a questionnaire between 01/11/2023 and 15/11/2023. 20 questions were designed under the following categories: demographics, HCC surveillance, general US LI-RADS, US technique, US LI-RADS category and US LI-RADS visualisation scores. Fisher's exact test was used to compare categorical data between the two hospitals.

**Results:** 26 practitioners completed the questionnaire [13 sonographers and 13 doctors]. 8 (30.8%) responses were from hospital 1 (US LI-RADS experience) and 18 (69.2%) from hospital 2 (no US LI-LIRADS experience). Overall, 39.2% of responses were "I don't know" for hospital 2 compared with 6.3% for hospital 1 ($p < 0.0001$). Correct responses were significantly higher for hospital 1 than hospital 2 overall (59.6% vs 20.1%, $p < 0001$) as well as for the following categories: general US LI-RADS (68.8% vs 30.6%, $p = 0.0168$), ultrasound technique (58.3% vs 24.1%, $p = 0.0048$), US LI-RADS category (62.5% vs 12.5%, $p < 0.0001$), and US LI-RADS visualisation score knowledge (56.3% vs 8.3%, $p = 0.0004$). There was no significant difference for HCC surveillance knowledge.

**Conclusion:** Although US LI-RADS knowledge was higher in the hospital with established US LI-RADS service, there were knowledge gaps for both hospitals in each category.

# PO3-02
# Volumetry-based assessment of post-hepatectomy liver failure

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**Background and aims:** Post-hepatectomy liver failure (PHLF) is a significant complication after liver resections, especially in major resections. Due to frequent lethal outcomes and limited therapeutic options, which are mostly limited to supportive therapies, the risk of severe PHLF should be evaluated preoperatively. The aim is to analyze if the volume of the future liver remnant (FLR), an objective, quantifiable measure, is a risk factor for liver resections.

**Method:** A retrospective analysis of major liver resections (hemihepatectomy and extended hemihepatectomy) for malignant and benign causes at Heidelberg University Hospital (2017-2021) was performed to investigate the predictive value of the volume of the FLR for clinically relevant PHLF, defined as grade B and C using the definition proposed by the International Study Group of Liver Surgery (ISGLS).

**Results:** 309 patients were included in the study. 270 patients (87 %) underwent surgery for malignant causes, 39 patients (13 %) for benign causes. Main malignant indications were colorectal liver metastases (n = 99; 32 %), cholangiocarcinoma (n = 94; 30 %) and hepatocellular carcinoma (n = 31; 10 %). 99 patients underwent left-sided resection, 210 patients right-sided resection. 33 % of patients (n = 103) developed clinically relevant PHLF. Blood loss, duration of surgery and diagnosis of extrahepatic cholangiocarcinoma were significant predictors for PHLF B/C in right- and left-sided resections (all p <0.002). The model of end-stage liver disease (MELD-score) also showed a significant correlation with PHLF B/C in all resections. For right-sided resections, an expected FLR of less than 30% emerged as the strongest predictive factor for PHLF B/C in multivariate regression analysis, which was associated with a 3.4 times higher risk of liver failure (HR = 3.390; 95% CI = 1.636-7.026; p = 0.001). Left-sided resections did not show this trend.

**Conclusion:** The volume of the FLR is as a significant predictor for clinically relevant liver failure in right-sided resections. Therefore, determination of the FLR should be routinely performed for right-sided major hepatectomies to identify patients at risk for clinically relevant PHLF. Further multicentric studies should validate these results, in addition to assessing the predictive power of FLR in non-standard major hepatectomies, e.g. after vascular embolization or in two-step hepatectomies. Based on our data and analysis, no recommendation for routine preoperative volumetry can be made for left-sided major resections.

# PO3-04-YI
# Analytical and clinical evaluation of a novel enzyme-immunoassay for the measurement of circulating protein induced by vitamin K absence or antagonist II in patients with hepatocellular carcinoma of viral-etiology

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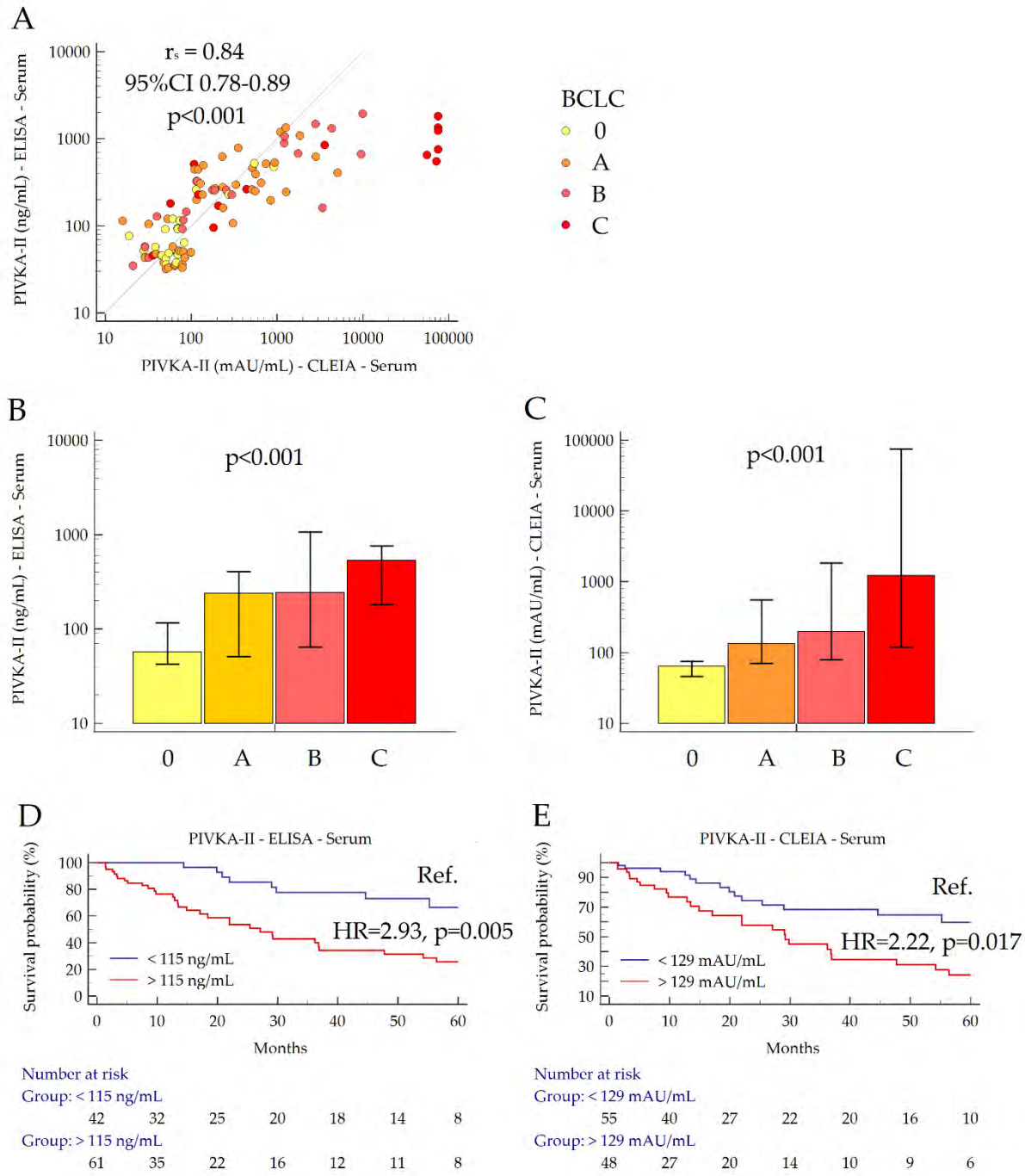
**Background and aims:** Hepatocellular carcinoma (HCC) is one of the most common cancers in the world, with high incidence and mortality. Protein induced by vitamin K absence or antagonist II (PIVKA-II) is an aberrant prothrombin secreted by hepatocytes due to impaired vitamin K-dependent carboxylase activity. Although the role of PIVKA-II in HCC is not entirely clear, the results of several studies support the use of PIVKA-II to monitor treatment outcomes and predict prognosis. To date, several standardized methods for PIVKA-II quantification are available, but they require automated and expensive platforms. The aim of this study was to evaluate the analytical and clinical performance of a novel enzyme-immunoassay (ELISA) for the measurement of serum PIVKA-II in patients with HCC in comparison to a consolidated chemiluminescence enzyme immunoassay (CLEAI) as reference test.

**Method:** We retrospectively enrolled 103 patients with HCC of viral-etiology (median age: 65 [57.0-71.7] years; males: 77 [74.8%]; HCV treated with DAA: 66 [64.1%]; HBV under Nuc treatment: 37 [35.9%]). HCC was classified according to Barcelona Clinic Liver Cancer (BCLC) staging system; 21 (20.4%) patients were BCLC 0, 46 (44.7%) BCLC A, 22 (21.4%) BCLC B, and 14 (13.6%) BCLC C. Median patients' follow-up after HCC diagnosis was 16.8 (7.7-49.2) months. Serum PIVKA-II was measured by ELISA (AssayGenie, Dublin, Ireland) and by CLEIA on the Lumipulse® G600 II platform (Fujirebio Inc., Tokyo, Japan); the corresponding results were reported as ng/ml and mAU/ml, respectively.

**Results:** In our cohort, median serum PIVKA-II values were 118 ng/ml by ELISA and 197 by CLEIA. The two methods showed a satisfactory correlation, with $r_s$ = 0.84, 95%CI 0.78-0.89, p <0.001 (Figure 1A). The conversion factor was calculated as the ratio between the measurements of the ELISA and CLEIA assays in each sample; the median was 0.87, and the interquartile range was 0.53-1.63. Furthermore, we investigated the association of PIVKA-II levels measured by ELISA and CLEIA with tumor burden and patients' overall survival (OS). ELISA and CLEIA PIVKA-II values distinctly increased according to BCLC stage (both p <0.001) (Figure 1B and C). By multivariate logistic regression analysis adjusted for BCLC stage, both ELISA PIVKA-II >115 ng/ml (Youden index) and CLEIA PIVKA-II >129 mAU/ml (Youden index) resulted significantly and independently associated to OS (HR = 2.93, 95%CI 1.38-6.21, p = 0.005 and HR = 2.22, 95%CI 1.15-4.27, p = 0.017, respectively) (Figure 1D and E).

**Conclusion:** ELISA PIVKA-II results in serum of patients with HCC correlated to those obtained with the reference CLEIA test. Furthermore, the two methods showed comparable clinical performance. The novel AssayGenie ELISA could represent a valid alternative to CLEIA assays for the measurement of PIVKA-II in serum, especially in resource-limited healthcare settings.

**Figure:**


A
rs = 0.84
95%CI 0.78-0.89
p<0.001
PIVKA-II (ng/mL) - ELISA - Serum
PIVKA-II (mAU/mL) - CLEIA - Serum
BCLC
0
A
B
C
B
p<0.001
C
p<0.001
D
PIVKA-II - ELISA - Serum
Survival probability (%)
Ref.
HR=2.93, p=0.005
< 115 ng/mL
> 115 ng/mL
Months
Number at risk
Group: < 115 ng/mL
42 32 25 20 18 14 8
Group: > 115 ng/mL
61 35 22 16 12 11 8
E
PIVKA-II - CLEIA - Serum
Ref.
HR=2.22, p=0.017
< 129 mAU/mL
> 129 mAU/mL
Number at risk
Group: < 129 mAU/mL
55 40 27 22 20 16 10
Group: > 129 mAU/mL
48 27 20 14 10 9 6

# PO3-09-YI
# Comparative analysis of subclassification models in patients with intermediate stage hepatocellular carcinoma (BCLC B) receiving systemic therapy

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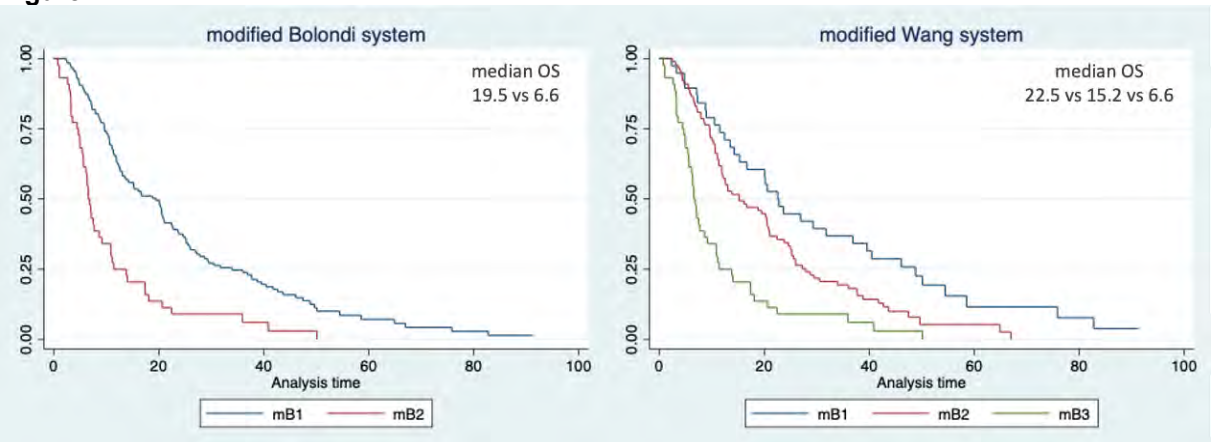
**Background and aims:** Intermediate stage hepatocellular carcinoma (BCLC B HCC) is a heterogeneous group of patients that could be addressed to a wide spectrum of treatments and, consequently, survival significantly varies among patients. In the last decades, several subclassification systems have been proposed to stratify patients' prognosis. We analyzed and compared these systems (Bolondi, Yamakado, Kinki, Wang, Lee, and Kim criteria) in patients undergoing systemic therapy.

**Method:** We considered 171 patients with BCLC B HCC treated with sorafenib as first-line systemic therapy in six different Italian centers from 2010 to 2021 and retrospectively applied criteria of six different subclassification systems.

**Results:** Except for Yamakado criteria, all the subclassification systems showed a statistically significant correlation to overall survival (OS). In the postestimation analysis Bolondi criteria (OS of subgroup 22.5, 11.9 and 6.6 mo, respectively; C-index 0.586; AIC 1338; BIC 1344) and Wang criteria (OS of subgroups 20.6, 11.9 and 7.0, respectively; C-index 0.607; AIC 1337; BIC 1344) presented the best accuracy. Further analyses on these two subclassification systems implemented with the prognostic factor of alpha-fetoprotein (AFP) >400 ng/ml have shown an increase of accuracy for both systems (C-index 0.599 and 0.624, respectively).

**Conclusion:** Intermediate stage subclassification systems maintain their predictive value also in the setting of systemic therapy. Bolondi and Wang criteria showed the highest accuracy. AFp >400 ng/ml enhance the performance of these systems.

**Figure:**



# PO3-10
# Locoregional control of proton beam therapy for hepatocellular carcinoma with extrahepatic lymph node metastasis

Takashi Iizumi[1], Toshiyuki Okumura[2], Hirokazu Makishima[1 3], Masahiko Harada[1], Den Fujioka[1], Hazuki Nitta[1 4], Haruka Shirataki[1 2], Keiichiro Baba[1], Motohiro Murakami[1], Toshiki Ishida[1], Masatoshi Nakamura[1], Takashi Saito[1 3], Haruko Numajiri[1 3], Masashi Mizumoto[1 3], Kei Nakai[1 3], Hideyuki Sakurai[1 3]

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**Background and aims:** While guidelines suggest systemic therapy for Barcelona Clinic Liver Cancer (BCLC) stage C hepatocellular carcinoma (HCC), the importance of locoregional control for intrahepatic lesions, especially those with portal invasion, has been reported even in these patients. However, the impact of locoregional control in patients with extrahepatic metastasis (EHM) of lymph node (LN) has not been elucidated in the era of precision radiotherapy. Here, we investigated the locoregional control and survival of HCC patients with LN metastasis.

**Method:** This single-institutional retrospective study identified 16 patients with HCC underwent PBT both intrahepatic primary legion and EHM of LN from September 2009 to November 2019 from an all-in treatment database. Survival curves were estimated by Kaplan-Meier method. Portal vein tumour thrombosis (PVTT) in the first branch or main trunk of the portal vein and inferior vena cava tumour thrombosis (IVCTT) were classified into major vascular invasion (MVI).

**Results:** The median follow-up time was 12 months (range, 4-64 months). MVIs were observed in six (37.5%) patients (PVTT in four patients and IVCTT in two patients), respectively. The median size of the primary tumour and the metastatic lymph node were 6.8 cm (IQR, 4.5-10.3) and 2.1 cm (IQR, 1.4-3.1). Among all 16 lymph node metastases, 13 were resided in reginal area (six para-aortic and four hepato-duodenal lymph regions) while three were in extra-regional area (an anterior mediastinal lymph node, a cardiophrenic lymph node and a pancreatic lymph node). The goals of treatment were radical for nine patients (81.8%) and locoregional control for two (18.2%). The 1- and 2-year cumulative incidences of local recurrence of primary lesions and EHM of LNs were 18.5% and 18.5%, and 0.0% and 16.7%, respectively. The 1- and 2-year overall survival rates and progression-free survival rates were 47.4% and 40.6%, and 35.2% and 23.4%, respectively.

**Conclusion:** Highly effective locoregional control of PBT for EHM of LN and primary disease was observed in this cohort. Given the potential synergistic effect, further studies exploring the combination of systemic therapy including immune-checkpoint inhibitors with PBT are anticipated to explore even more survival benefits for patients with advanced HCC.

# PO3-11-YI
# Risk of malignancy in patients with liver transplantation

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**Background and aims:** Liver transplantation is a life-saving treatment for end-stage liver failure and hepatocellular carcinoma (HCC). Patients are at risk for malignancy due to their prolongation in survival and, therefore, more exposure to immunosuppressive treatments. In our study, we aimed to evaluate the malignancy risk in liver transplant patients followed up gastroenterohepatology outpatient clinic from a tertiary center.

**Method:** The data of 348 patients who had liver transplantation were analyzed retrospectively from the hospital registry system. Descriptive statistics for the variables in the study; expressed as mean, standard deviation (SD), number (n) and percentage (%). “Independent T-test” was used to compare continuous measurements according to “categorical groups”. Chi-square test was calculated to determine the relationships between categorical variables.

**Results:** Of the total 348 patients, 135 (38.8%) were women, and 213 (61.2%) were men. The average age of the patients was 52.11 ± 17 years, and the average follow-up period was 149.19 ± 73.92 months. 23 (6.6%) patients, 15 men and 8 women, developed malignancy after liver transplantation. Of the patients with posttransplant malignancy, 14 were alive and 9 were cadaveric transplants (p = 0.075). The average age of patients who developed posttransplant malignancy was 59.22 ± 13.36 years, and the average follow-up period was 152.26 ± 62.67 months. The most commonly used immunosuppressive agent was tacrolimus (77%). There was a history of rejection in 19 patients. 57 (16.4%) patients had pre-transplant HCC, and patients with pre-transplant HCC had a higher risk of post-transplant malignancy than those without (p = 0.028). While the risk of malignancy after liver transplantation increased with age (p = 0.038), no correlation was found between the duration of liver transplantation and the risk of posttransplant malignancy. Immunosuppressives used after liver transplantation and a history of rejection were not statistically significant in terms of posttransplant malignancy risk. Hematological and lymphoproliferative disease was observed in 6 of 23 patients who developed posttransplant malignancy, skin malignancies in 8 (3 squamous cell carcinomas, 1 mycosis fungoides, 4 basal cell carcinomas), metastatic HCC in 4 patients, bronchial lung tumor in 1 patient, papillary thyroid cancer in 1 patient, papillary urothelial carcinoma in 1 patient, meningioma in 1 patient, and gastric cancer in 1 patient.

**Conclusion:** The incidence of posttransplant malignancy in liver transplant patients was 6.6% and the most common malignancy was skin cancer. Older age and pre-transplant HCC is an important risk factor for post-transplant malignancy.

# PO3-15-YI
# Time-trends in cholangiocarcinoma mortality-a Danish nationwide cohort study

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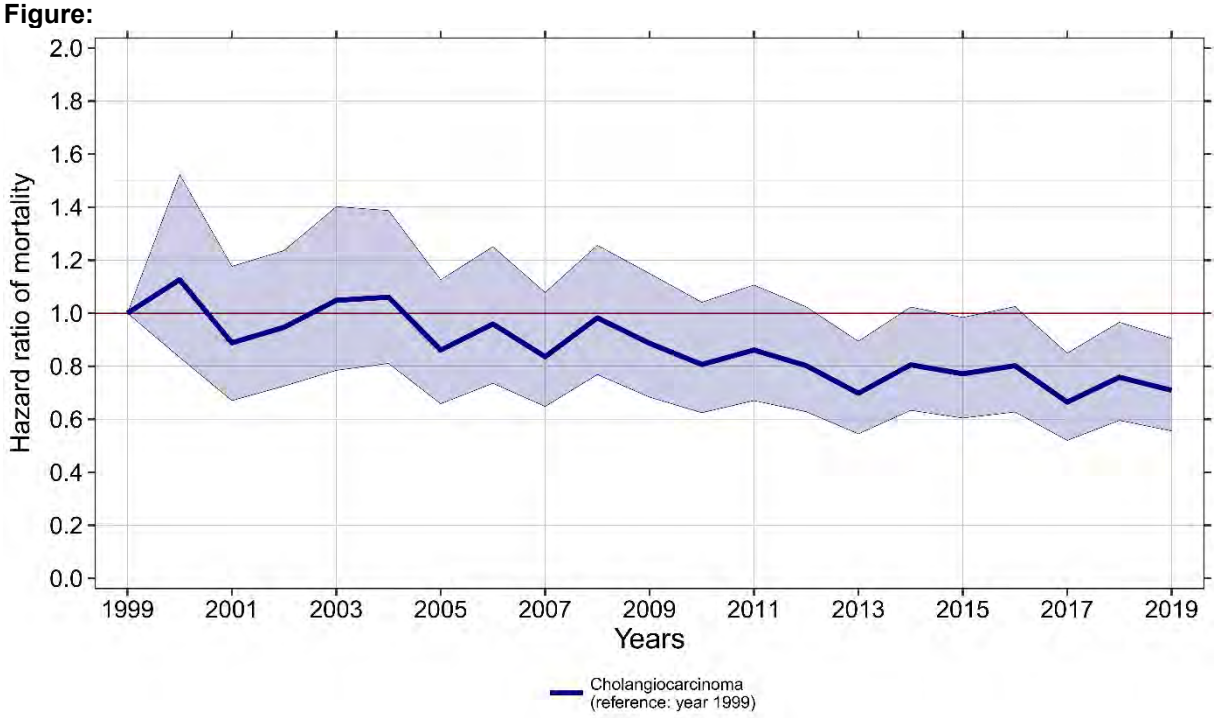
**Background and aims:** Cholangiocarcinomas (CCA) originate from the biliary epithelium and have a poor prognosis; the incidence of CCA is rising in Denmark. It is crucial for our understanding of the disease to examine mortality, so we set out to examine time trends in mortality of CCA in a nationwide Danish cohort.

**Method:** We included all 3, 260 Danish patients with a diagnosis of CCA (ICD-10 codes C22.1, C24.0, C24.8, C24.9) in 1999-2019 recorded in both the Danish Cancer Registry and the National Patient Registry. We computed 1-year mortality dependent on year of CCA diagnosis. We used Cox proportional hazards regression to estimate adjusted mortality hazard ratios (HR) for sex, age, year of diagnosis and type of CCA.

**Results:** Of the 3, 260 CCA patients, 47% were men. The median age of patients diagnosed in 1999 was 72 vs. 71 in 2019. The 1-year mortality decreased between 1999 and 2019 from 76.5% (95% confidence interval 67.9-84.2) in 1999 to 68.0% (61.8-74.1) in 2019 and adjusted mortality HRs were: female sex 0.97 (0.90-1.04), age [10-year increase] 1.29 (1.25-1.34), for intrahepatic CCA vs. unspecified CCA 0.77 (0.70-0.85), for extrahepatic CCA vs. unspecified CCA 0.66 (0.60-0.73), and for year of diagnosis HRs decreased with more recent diagnosis year. Adjusted mortality HRs for year of diagnosis are shown in the figure.

**Conclusion:** The 1-year mortality of CCA has decreased between 1999 and 2019. Mortality decreased with more recent year of diagnosis after confounder adjustment, though 1-year mortality remained high.

**Figure:**



# PO4-02
# Cholangiocarcinoma across England: evidence of regional, socioeconomic, and temporal variation in incidence, survival, routes to diagnosis and treatment

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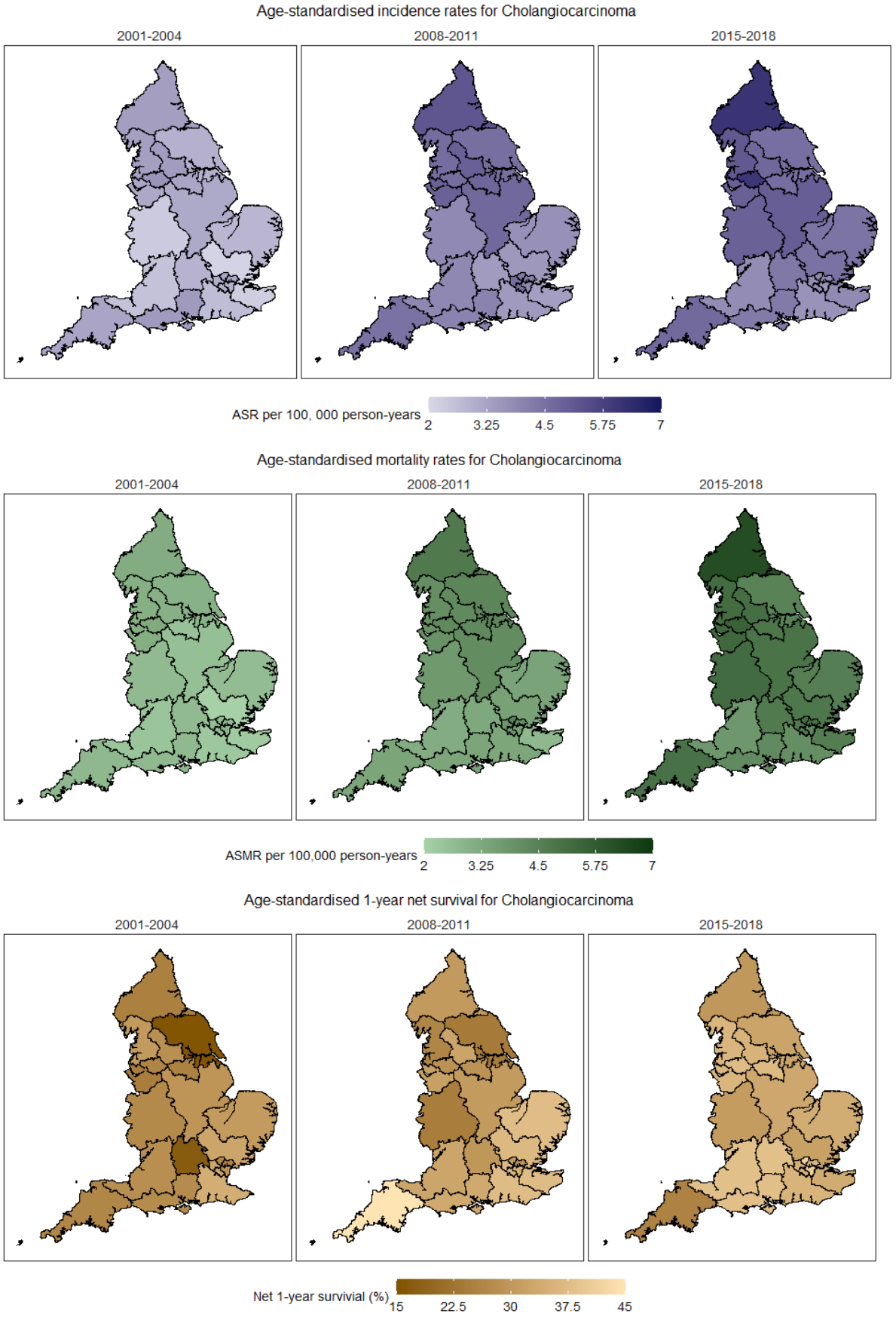
**Background and aims:** Cholangiocarcinoma (CCA) is increasing in the UK and globally. Whether there are regional, socioeconomic and temporal variations in incidence, mortality, routes to diagnosis (RtD) and treatment within England is unknown. There are no published studies from any country exploring these issues. This work aimed to investigate these issues across England.

**Method:** Patients diagnosed in England with a CCA between 2001-2018 were extracted from the National Cancer Registration Dataset (NCRD) linked to other data sources: the Hospital Episode Statistics (HES) dataset, the Systemic Anti-Cancer Therapy (SACT) dataset. RtD data were extracted for 2006-2017, and treatment data for 2014-2017, representing the time periods for which respective data were available. Age-standardised incidence (ASRs) and mortality (ASMRs) rates were estimated overall and stratified by gender, area deprivation quintile based on the income domain of the Index of Multiple Deprivation closest to diagnosis, tumour subtype (intra/extrahepatic) and England's 21 regional Cancer Alliances. Net and overall survival estimates were also calculated. Linear probability models were used to quantify geographic variation in RtD and treatment, adjusting for potential confounders. Geographic variation was analysed by Cancer Alliance based on postcode of residence at diagnosis.

**Results:** ASRs, ASMRs and net survival varied by geography. ASRs and ASMRs were consistently higher in the more socioeconomically deprived groups. The least deprived CCA patients had best overall survival ($p < 0.001$). 3-year net survival rose from 9.2% in 2001-2003 to 12.6% by 2016-2018. The most common RtD was Emergency Presentation (EP), with 49.6% of patients diagnosed thus. The proportion diagnosed via Two Week Wait Urgent GP Referral (TWW) doubled from 9.9% to 19.8%. Statistically significant variation was observed in the proportions of patients diagnosed via a TWW or EP across England's Cancer Alliances. There was also significant variation between Cancer Alliances in the proportions of patients that received potentially curative surgery, systemic therapy without potentially curative surgery, or stent insertion without potentially curative surgery.

**Conclusion:** Geographic variations in RtD suggest there are opportunities for sharing of good practice across NHS organisations to continue to improve on current trends. Local review of treatment pathways and more comprehensive guidelines for the management of CCA could help to reduce potential variation in treatments received across Cancer Alliances. Whilst there were some improvements in overall survival over time, three-year survival rates remained extremely low, highlighting huge unmet need in this population.

**Figure:** Regional and Temporal variation in Cholangiocarcinoma age-standardised incidence and mortality rates, and 1-year net survival by Cancer Alliance



# PO4-05
# Impact of glucose homeostasis on postoperative complications after major hepatectomies

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**Background and aims:** There is limited evidence on the impact of serum glucose concentration on complications after liver resection. Liver plays a central role in coagulation, immune function, and metabolism, and is particularly sensitive to metabolic dysfunction. This study investigates the relationship between blood glucose concentration and complications after major hepatectomies.

**Method:** Data from 331 patients after major liver resection (three or more segments) between 2017 and 2022 were retrospectively analyzed at Heidelberg University Hospital. Chi-square test, Mann-Whitney U test, logistic regression, and Kaplan-Meier curves were used to examine the effects of preoperative as well as early postoperative hyperglycemia (within four hours after surgery) on postoperative complications.

**Results:** Preoperatively, 57 patients (17.2%) and postoperatively, 177 patients (53.5%) showed hyperglycemia (>125 mg/dl). Postoperative complications occurred in 231 patients (morbidity, 69.8%): 79 patients (23.9%) had mild complications (Clavien-Dindo grade I+II) and 152 patients (45.9%) experienced severe complications (Clavien-Dindo grade IIIa-V). Patients with preoperative hyperglycemia showed an increased risk of postoperative sepsis ($p = 0.038$), severe bile leakage requiring surgery (grade C) ($p = 0.012$), and an increased risk of re-laparotomy ($p = 0.031$). Patients with postoperative hyperglycemia had an increased risk of severe complications ($p = 0.036$).
Multivariate analysis identified preoperative hyperglycemia as an independent predictor for biliary leakage requiring surgery (grade C) (OR = 2.881; 95% CI: 1.302-6.375; $p = 0.009$) and the need for re-laparotomy (OR = 2.152; 95% CI: 1.090-4.248; $p = 0.027$).

**Conclusion:** Hyperglycemia is a cardinal sign of a major comorbidity. This study illustrated the negative impact of hyperglycemia on postoperative complications after major hepatectomy, including increased risk of severe complications and repeat surgery. Perioperative glucose screening to identify patients at risk should be implemented and impact of strict perioperative glucose control after hepatectomy on complication rates should be studies. Studies aiming to identify predictors and triggers of perioperative hyperglycemia, e.g. intraoperative stress, should be initiated.

# PO4-06-YI
# Sorafenib as a second-line treatment after failure of atezolizumab-bevacizumab

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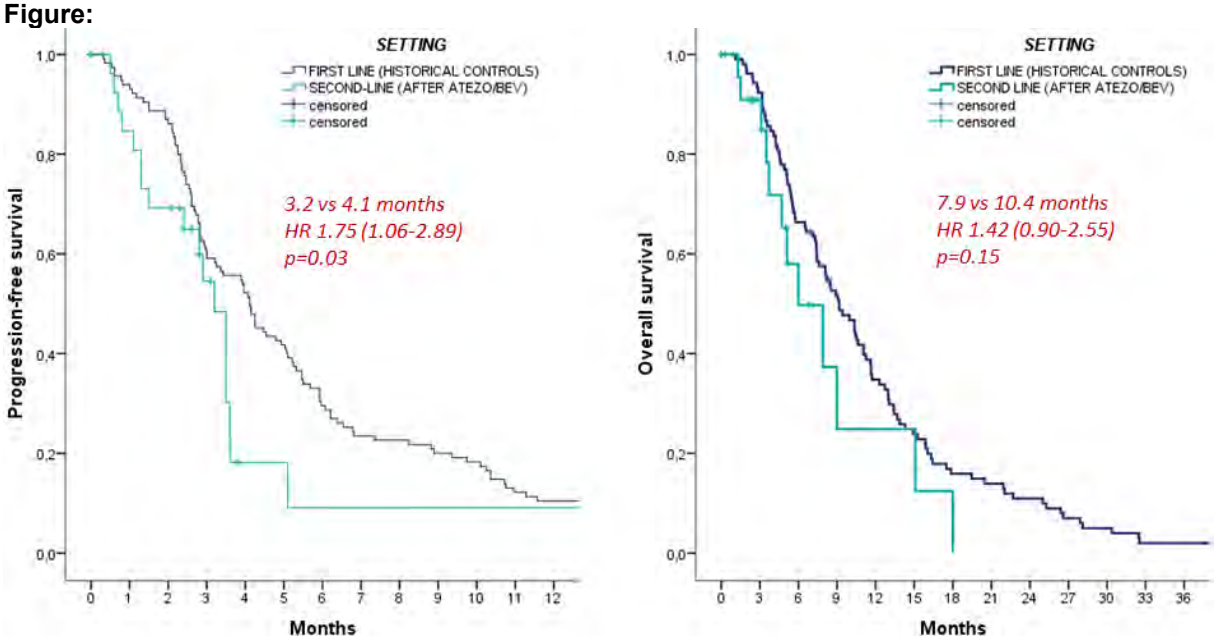
**Background and aims:** Patients receving atezolizumab/bevacizumab (AB) for HCC may have a primary resistance to this combination. Another relevant proportion of patients will develop a secondary resistance and, eventually, experience progression disease. Randomized clinical trials (RCTs) trying to identify second-line treatments are undergoing. If such trials are not available or patients are non-eligible, sorafenib is often prescribed, based on European approval and reimbursement policies. However, evidence supporting these policies is lacking, as no RCTs explored sorafenib in this setting.We aimed to assess the efficacy of sorafenib in patients who permanently stopped AB.

**Method:** The ARTE database collects prospectively enrolled patients treated with AB in a real-life setting (March 2022-November 2023). We analysed the outcome of patients who received sorafenib as second-line treatment. Moreover, we performed a case-control matching with historical controls from the ARPES database (n = 712) who had received sorafenib as a frontline treatment before AB had become available in clinical practice. Patients were matched 4:1, based on known predictor of overall survival (OS) in sorafenib-treated patients (Child-Pugh class, AFp >400ng/ml, macrovascular invasion, extrahepatic spread, ECOG-PS>0).

**Results:** Amongst the 157 patients included in the ARTE database, 130 (67.4%) permanently discontinued AB. Of them, 57 received a second-line treatment. Sorafenib was prescribed in 29 patients. The disease control rate (DCR) was 17.2%, with no objective responses. The median PFS and OS were 3.2 and 7.9 months. When compared with historical controls, patients who received sorafenib as a second-line therapy had worse DCR (17.1 vs 47.4%, p <0.01) and PFS. A trend toward a worse OS was also noted (Figure)

**Conclusion:** In the post-AB setting, we found suboptimal efficacy outcome of sorafenib. The very low DCR suggest that resistance to AB might select tumour cells able to escape the therapeutic targets of sorafenib. Enrollment of these patients in RCTs is strongly recommended to identify better therapeutic strategies.

**Figure:**


SETTING
FIRST LINE (HISTORICAL CONTROLS)
SECOND-LINE (AFTER ATEZO/BEV)
censored
censored
3.2 vs 4.1 months
HR 1.75 (1.06-2.89)
p=0.03
Progression-free survival
Months
SETTING
FIRST LINE (HISTORICAL CONTROLS)
SECOND LINE (AFTER ATEZO/BEV)
censored
censored
7.9 vs 10.4 months
HR 1.42 (0.90-2.55)
p=0.15
Overall survival
Months

# PO4-07-YI
# Bile extracellular vesicles hold protein biomarkers for the early diagnosis of cholangiocarcinoma in individuals with primary sclerosing cholangitis

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**Background and aims:** Cholangiocarcinoma (CCA) presents a significant threat to individuals with primary sclerosing cholangitis (PSC), with a 20-year cumulative incidence of approximately 15%. Early diagnosis is challenging due to overlapping symptoms, and recommended MRI/MRCP surveillance every 6-12 months often proves suboptimal in detecting early-stage cancer. PSC-CCA patients face a grim prognosis, with a median overall survival of 5-12 months in unresectable cases, making CCA the primary cause of PSC-associated mortality. There is a critical need for more accurate early detection methods, allowing access to potentially curative options like tumor resection or liver transplantation. In this regard, investigating extracellular vesicles (EVs) in bile, which come into direct contact with CCA tumors, offers a promising avenue for identifying diagnostic CCA biomarkers in PSC, and these were evaluated in this study.

**Method:** Bile EVs were collected from patients with isolated PSC (PSC, n = 52), PSC with CCA (PSC-CCA, n = 14), or PSC at time of sampling but who later developed CCA (PSC to CCA, n = 8), at Oslo University Hospital Rikshospitalet (Norway). The EV-protein content was characterized using mass spectrometry. Diagnostic biomarkers for PSC-CCA, as well as early-diagnostic/predictive biomarkers for the PSC to CCA group were identified and combined using binary logistic regression multivariable models.

**Results:** High-throughput proteomics of bile EVs identified 21 diagnostic biomarkers for PSC-CCA, regardless of sex, age, the presence of inflammatory bowel disease, or cirrhosis at the time of sampling. Among these, 14 biomarkers were observed to be more abundant, and 7 exhibited lower levels in patients with PSC-CCA compared to patients with isolated PSC. Machine learning algorithms revealed COPA/ATP5H/VTNC/IQGA1/PRDX2 (AUC = 0.996) and COPA/ATP5H/VTNC/IQGA1/CALX/PRDX2 (AUC = 1.000) as highly effective in diagnosing PSC-CCA versus isolated PSC, surpassing the performance of serum CA19-9 alone (AUC = 0.846). Notably, the logistic model combining TM9S4/RS18/LPPRC/NHRF1 demonstrated predictive capacity for CCA development in PSC before any clinical evidence of malignancy with 100% sensitivity and specificity (AUC = 1.000), whereas serum CA19-9 exhibited no significant predictive capacity for CCA development (AUC = 0.596).

**Conclusion:** Bile EVs harbor valuable protein biomarkers for predicting the development of CCA and enabling early diagnosis in individuals with PSC. Given the ease of bile collection during stenting for dominant strictures in individuals with PSC, this innovative liquid biopsy tool may be of significant value for monitoring disease progression and aiding access of potentially curative treatment options.

# PO4-13-YI
# The correlation between Interleukin-6 and the progression of chronic hepatitis B

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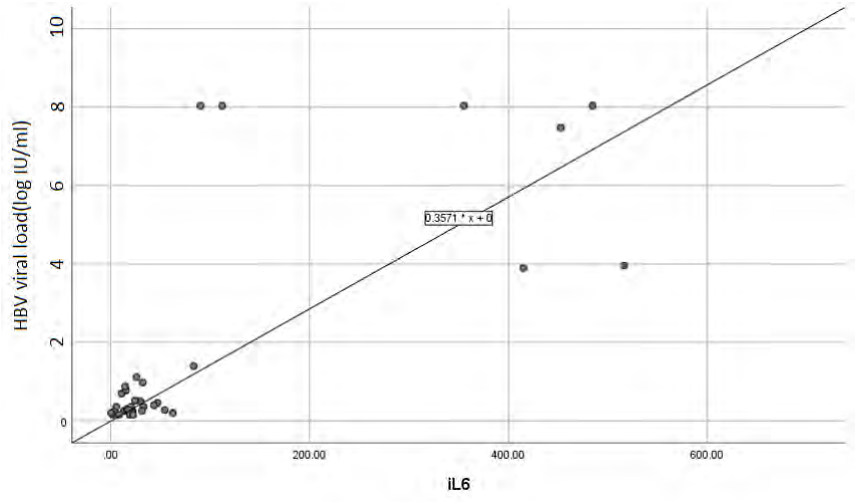
**Background and aims:** Hepatitis B virus (HBV) is a dreadful virus with the potential to cause human liver diseases such as self-limiting acute hepatitis, chronic hepatitis, fulminant hepatic failure, liver cirrhosis and hepatocellular carcinoma (HCC). These complications resulted from an immune response of the host that affects both outcome and disease progression, rather than a direct cytopathic effect. Cytokines have been shown to be engaged in regulating hepatocyte functions, and play an important role in HBV infection immunopathogenesis.

**Method:** About 52 subjects ranging from 18 years old to 80 years old that were diagnosed with HBV were recruited into the studies. Their venous blood was taken and centrifuged at 4500rpm for 5 minutes to separate the blood components. The patient's sera were withdrawn and divided into two aliquots and kept in a special fridge with a temperature of -20 to -70 degrees. The first group of sera were subjected to a hybrid capture, tube-based signal amplification using HBV Digene Hybrid-Capture I, Digene Corporation, USA. While the second group of sera were subjected to a sandwich-ELISA test using LEGEND MAX Deluxe set human IL-6 kit to quantify the IL-6 levels. Both data were recorded and analysed using IBM SPSS version 26 software.

**Results:** We found that there was a direct correlation between the severity of HBV viral load and the level of IL-6. The more severe the infection, the higher the IL-6 level ($p < 0.05$) taking the mean value of IL-6 as 132.6pg/ml. Demographical data distributions showed that men, aged between 40-60years old and healthcare workers were the risk factors to develop chronic HBV. A linear scatter plot was derived between the levels of IL-6 and HBV viral load. Pearson correlation coefficient showed a linear correlation between the two variables. The patient's ALT enzyme was used to stratify the severity of the liver functions. Higher levels of IL-6 were detected in the subjects with HBV for longer than 6 months which proved that IL-6 levels correspond to the chronicity of the disease.

**Conclusion:** IL-6 is a vital mediator of inflammation and the acute phase response of the liver. Our studies proved that serum IL-6 levels were positively correlated with HBV disease severity and chronicity. Thus, IL-6 may be a useful indicator of disease activity and therapeutic efficacy in patients suffering from hepatitis B.

**Figure:**



# PO4-14-YI
# Identification of factors associated with primary refractoriness to atezolizumab plus bevacizumab in patients with advanced hepatocellular carcinoma

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**Background and aims:** Despite showing an unprecedented survival benefit against sorafenib, atezolizumab plus bevacizumab (A+B) is not universally efficacious in patients with advanced hepatocellular carcinoma. Primary refractoriness to A+B is associated with dismal prognosis. However, mechanisms underscoring lack of response are poorly understood.

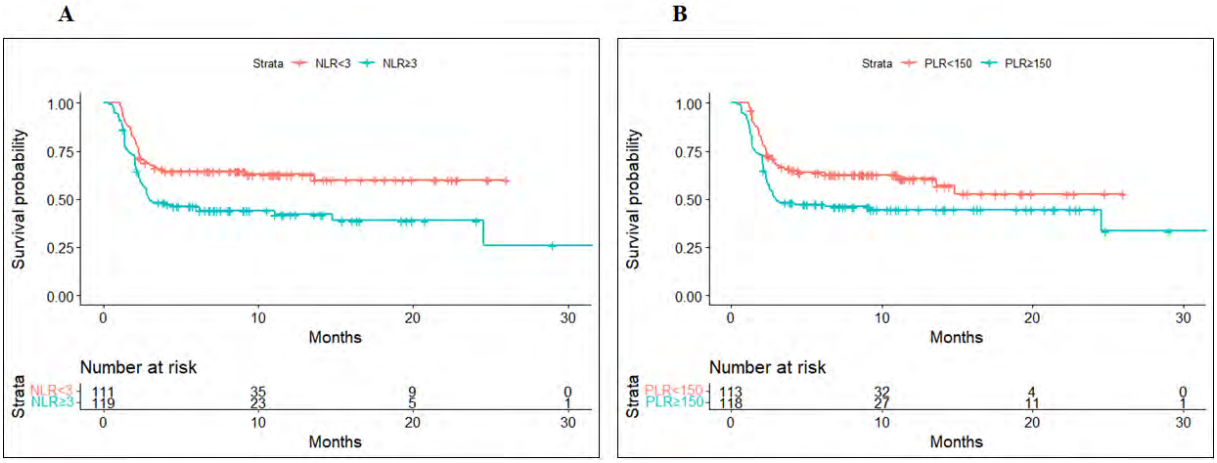
**Method:** From a large multinational dataset of 901 patients belonging to 24 centers, we identified a subgroup of 591 patients with Child-Pugh grade A who received A+B as first-line systemic therapy. We stratified the cohort in “primary progressors (PP), i.e. patients with progressive disease (PD) at the first

radiological assessment after therapy start and “responders” (RE), i.e. patients with either partial response (PR) or complete response (CR), excluding patients with stable disease. Baseline characteristics of the two groups, comprising neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) as expression of systemic inflammation, were analysed with Mann-Whitney U test and two-tailed χ2 test where appropriate. Clinical factors associated with patients’ response to therapy were used to stratify OS and PFS by Kaplan-Meier methodology, followed by Log-rank test in univariate analysis.

**Results:** Baseline characteristics of the entire cohort were analysed. Median OS was 16.8 months (95% confidence interval [CI] 14.7-18.9) and median PFS was 8.2 months (95% CI 6.9-9.4). Considering the characteristics of PP (171 patients) and RE (125 patients), no differences between the two groups were underlined regarding sex, age, liver disease aetiology, Child-Pugh grade, macrovascular invasion, extrahepatic disease spread and albumin-bilirubin grade. The PP group was characterised by higher tumour burden based on Barcelona Clinic Liver Cancer (BCLC) stage (A-B 20.8%, C 79.2% vs A-B 31%, C 69% p = 0.047), higher NLR and PLR (medians 3.7 vs 2.6, p <0.001 and 168.6 vs 135.4, p = 0.008, respectively). NLR ≥3 was associated with shorter OS (12.9 months versus 24.2 months, p <0.001) and PFS (11.0 months versus 3 months, p = 0.006 respectively, **Panel A**). Similarly, PLR >150 predicted for shorter OS and PFS compared with those with PLR <150 (14.1 months versus 21.6 months, p = 0.01 and 3 months versus13.4 months, p = 0.004 respectively, **Panel B**). No statistically significant difference was found among patients with BCLC A or B versus C both for OS and PFS.

**Conclusion:** Higher levels of systemic inflammation were associated with primary refractoriness to A+B, shorter PFS and poor OS outcome regardless the disease stage. Modulation of the systemic inflammatory status might augment responsiveness to A+B in this prognostically disadvantaged population.

**Figure:**



# PO4-15-YI
# Evaluation of overall survival using restricted mean survival time in advanced biliary tract cancer treated with immunotherapy: systematic review and meta-analysis

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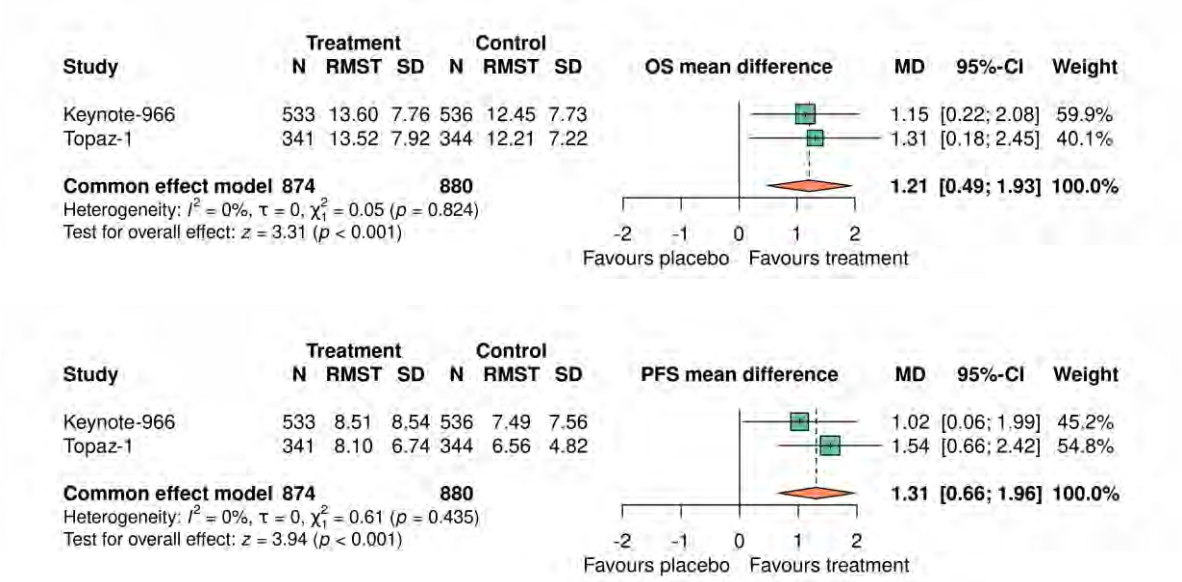
**Background and aims:** In biliary tract cancer (BTC), the addition of immunotherapy (durvalumab or pembrolizumab) to gemcitabine and cisplatin (GemCis) has significantly improved overall survival (OS) in phase 3 clinical trials (RCTs). However, the interpretation of the results is challenging because OS and progression-free survival (PFS) Kaplan-Meier curves violate the proportional hazards (PH) assumption. Analysis using the restricted mean survival time (RMST) allows quantification of the absolute benefit in the absence of PH. This systematic review and meta-analysis aim to quantitatively assess the benefit of immunotherapy-based regimens (IOs) for both OS and PFS at 24 months, using RMST analysis.

**Method:** Systematic searches were conducted using databases, clinical trial registries, and conference abstracts of studies published until November 8, 2023. Only phase 3 RCTs evaluating the use of anti-PD-1/PD-L1 combined with GemCis and reporting OS and PFS rates were included. KM curves for OS and PFS were digitized using WebPlotDigitizer v.4.6, and the data were reconstructed. A meta-analysis of extracted data for OS and PFS using RMST at 24 months was performed, employing common-effect models to estimate weighted average RMST and mean differences between treatment and control groups. Heterogeneity was assessed using the $I^2$ statistic.

**Results:** A total of 1, 754 participants from the KEYNOTE-966 and TOPAZ-1 trials were included. In the TOPAZ-1 study, RMST OS at 24 months was 13.52 (7.92) and 12.21 (7.22) months with GemCis plus durvalumab and GemCis, respectively. In the KEYNOTE-966 study, OS at 24 months was 13.60 (7.76) and 12.45 (7.73) months with GemCis plus pembrolizumab and GemCis, respectively. IOs regimens showed a mean OS difference at 24 months by RMST of 1.21 months [ (95% CI: 0.49-1.93), $p < 0.001$, $I^2 = 0\%$]. RMST PFS at 24 months in TOPAZ-1 was 8.10 (6.74) and 6.56 (4.82) months with GemCis plus durvalumab and GemCis, respectively. Similarly, in the KEYNOTE-966 study, it was 8.51 (8.54) and 7.49 (7.56) months with GemCis plus pembrolizumab and GemCis, respectively. The meta-analysis of mean PFS difference by RMST was 1.31 [ (95% CI: 0.66-1.96), $p < 0.001$, $I^2 = 0\%$] months in patients undergoing IOs.

**Conclusion:** Combining IOs with GemCis offers a significant benefit for the survival of patients with advanced BTC. With this magnitude of benefit, it is essential to weigh individual patient factors, preferences, and potential risks in therapeutic decision-making. RMST analysis provides valuable information to patients and physicians, facilitating decision-making in a value-based medical environment.

**Figure:**


Treatment
Control
Study N RMST SD N RMST SD
OS mean difference
MD 95%-CI Weight
Keynote-966 533 13.60 7.76 536 12.45 7.73 1.15 [0.22; 2.08] 59.9%
Topaz-1 341 13.52 7.92 344 12.21 7.22 1.31 [0.18; 2.45] 40.1%
Common effect model 874 880 1.21 [0.49; 1.93] 100.0%
Heterogeneity: $I^2$ = 0%, $\tau$ = 0, $\chi^2_1$ = 0.05 ($p$ = 0.824)
Test for overall effect: $z$ = 3.31 ($p$ < 0.001)
-2 -1 0 1 2
Favours placebo Favours treatment


Treatment
Control
Study N RMST SD N RMST SD
PFS mean difference
MD 95%-CI Weight
Keynote-966 533 8.51 8.54 536 7.49 7.56 1.02 [0.06; 1.99] 45.2%
Topaz-1 341 8.10 6.74 344 6.56 4.82 1.54 [0.66; 2.42] 54.8%
Common effect model 874 880 1.31 [0.66; 1.96] 100.0%
Heterogeneity: $I^2$ = 0%, $\tau$ = 0, $\chi^2_1$ = 0.61 ($p$ = 0.435)
Test for overall effect: $z$ = 3.94 ($p$ < 0.001)
-2 -1 0 1 2
Favours placebo Favours treatment

# PO4-16
# Improved multiphase liver CT scan quality with implementation of a new contrast protocol

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**Background and aims:** The aim of this quality improvement project was to assess the quality of CT liver images pre- and post-implementation of a bolus-tracked, weight-based intravenous (IV) contrast protocol.

**Method:** Two cohorts with 50 consecutive CT liver scans in each group were retrospectively compared (2021 group had a fixed IV contrast dose and fixed scan delay versus the 2023 group with a new weight based bolus tracking protocol). Patient demographics, IV contrast dose and contrast injection rate were extracted from our Radiology Information System. Quality of each CT liver was assessed subjectively by visually assessing if the correct phase of contrast was carried out and if lesion depiction quality was adequate on a 2 point scale. Quantitatively, for each CT, regions of interest were placed on the abdominal aorta, portal vein (PV) and liver parenchyma. Using SPSS, the two groups were compared using t test, Mann-Whitney U and Fisher's exact tests.

**Results:** There was no significant difference in age ($p = 0.08$), sex ($p = 0.81$) or weight ($p = 0.66$) between the two groups. Contrast dose ($p < 0.001$) and injection rate ($p < 0.001$) were significantly higher in the 2023 group. Both subjectively, appropriately timed arterial phase contrast (42/50 vs 32/50, $p = 0.039$), and quantitatively, the abdominal aorta ($p < 0.001$), PV ($p < 0.001$) and liver ($p < 0.001$) Hounsfield units, were significantly higher in the 2023 group. Adequate lesion depiction quality was also higher in the 2023 group (31/42 vs 8/39, <0.001).

**Conclusion:** The implementation of a bolus-tracked, weight-based IV contrast dosing CT liver protocol significantly improved image quality and lesion depiction.

# PO5-01
# Idarubicin-infused transarterial chemoembolization (TACTida) as potential therapy for HCC-patients : clinical and ex-vivo assessment of drug response and survival to varying IDA concentrations

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**Background and aims:** Hepatocellular carcinoma (HCC) is the most frequent type of primary liver cancer, and is often diagnosed in its advanced stages with underlying chronic liver diseases. Trans-arterial chemoembolization (TACE) is a locoregional treatment and is considered as the first-line cancer therapy for advanced HCC. It consists in administering chemotherapy at high-intensity concentrations via catheterization, targeted at the tumor-supplying hepatic arterial branch. Recent studies have shown that IDA presents similar effects than DOX as well as a more stable emulsion leading to enhanced membrane permeability. The purpose of this study is to investigate different concentrations of TACE-infused IDA to determine drug response through imaging, histopathology, and ex-vivo organoid culture. Treatment efficacy will be assessed in relation to HCC phenotype, characteristics, and tumor microenvironment.

**Method:** Patients with advanced-stage HCC were administered two different concentrations of IDA via TACE (10mg and 15mg). Blood samples, tissue and fluid biopsies, and PET/MRI scans were conducted before and after the initial TACE treatment of each patient. Biopsies from both non-tumor and tumoral liver parenchyma were taken, part being cultured ex-vivo to generate 3D model organoids.

**Results:** By processing PET/MR images of liver tumor, a difference in median area under the FDG dynamic uptake curve was observed between viable tumor and non-tumor tissues. Parallelly, the FDG uptake area showed a correlation with tumorous volume. Viable tumor volume measurements from MRI areas of interest diverged in relation to mRECIST tumor diameter. Six out of seven patients receiving both IDA regimens responded favorably to the higher concentration. HCC organoid cultures were subjected to several doses of IDA, while cell viability was assessed using CellTiter-Glo. IDA was able to dose-dependently decrease HCC organoid growth with half maximal inhibitory concentration (IC50) values that varied by 2.5- fold from 2.0 to 10.0 mM. Non-tumor and tumoral patient-derived Organoid viability was shown to be impaired following 24-hour exposure to 2.5 mM IDA. A difference in exposure to 2.5 mM IDA was detected between patient-derived non-tumoral and tumoral organoids. A higher level of IDA-resistance was seen in patients 23, 11, 10 and 7. Viable tumors were more responsive to IDA than non-tumoral organoids. IDA responders were determined with a viability rate under 74% after IDA treatment, while non-responders were defined by a viability rate over 80% after IDA treatment.

**Conclusion:** Both clinical and ex-vivo studies have respectively demonstrated differences in tumor viability. The further investigation of IDA response as well as resistance could provide promising perspectives for TACE therapy in advanced-stage HCC patients.

# PO5-04-YI
# Revolutionizing post-surgical outcome predictions: non-invasive tests and AI for hepatocellular carcinoma patients

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**Background and aims:** Hepatic resection is crucial for hepatocellular carcinoma (HCC) treatment, but precise patient selection based on factors like tumor size and portal hypertension is vital for accurate prognosis. This study aimed to assess serum liver tests' effectiveness in identifying clinically significant portal hypertension (CSPH) and predicting post-hepatectomy liver failure (PHLF), comparing them with liver stiffness measurement (LSM). A machine learning approach was also evaluated to develop and validate a mathematical model for PHLF.

**Method:** A cohort comprising 128 patients with compensated cirrhosis and hepatocellular carcinoma (HCC), who underwent hepatic resection at the Regional Institute of Gastroenterology and Hepatology Cluj-Napoca between 2016 and 2023 was included in the study. Clinically significant portal hypertension (CSPH) was defined as either a hepatic venous pressure gradient (HVPG) of ≥10 mmHg or the presence of esophageal varices, splenomegaly, and thrombocytopenia (<100, 000/mm3). Non-invasive serum tests were employed: APRI, FIB-4, NLR, eLIFT, and ALBI. The performance of these non-invasive tests in predicting CSPH and prognosis was evaluated using the area under the receiver operating characteristic (AUROC) curves. Logistic Regression, a machine learning algorithm, established a mathematical model for predicting decompensation.

**Results:** In the cohort under consideration (mean age: 65 ± 7 years; etiological distribution: 32% alcohol, 43% viral hepatitis C, 20% viral hepatitis B, and 5% other etiologies), 45% exhibited CSPH. Notably, APRI, FIB4, and eLIFT demonstrated commendable predictive capacity for CSPH (AUROC = 0.87, 95% CI: 0.79-0.95; p <0.05; AUROC = 0.88, 95% CI: 0.81-0.96; p <0.05; and AUROC = 0.83, 95% CI: 0.73-0.92; p <0.05, respectively). However, it is noteworthy that liver stiffness measurement (LSM) exhibited superior performance in predicting CSPH (AUROC = 0.913, 95% CI: 0.84-0.98; p <0.05). LSM, APRI, and FIB-4 exhibited a discernible trend in predicting PHLF. Using AI algorithms, we have determined the following equation for probability of PHLF: probability = 1/ (1+e^ (-1* (-3.4220+1.7835*ACLF_Grade+ 0.0796 *Hospitalisation_days +-0.5725*Number_of_Organ_Failures + -0.3648 * Vent+2.6405*bili)) with an LR accuracy of 0.937.

**Conclusion:** While liver stiffness measurement (LSM), APRI, FIB-4, and eLIFT demonstrate the capability to identify patients with clinically significant portal hypertension (CSPH) among those with hepatocellular carcinoma (HCC) undergoing hepatic resection, it is noteworthy that they lack the predictive capacity for prognosis in this clinical context. The essence of identifying patients susceptible to postoperative decompensation may be found in the elaboration of mathematical models employing artificial intelligence algorithms.

# PO5-05
# Real-world comparison of overall survival in patients with unresectable hepatocellular carcinoma treated with Folfirinox or Gemcitabine-Paclitaxel

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**Background and aims:** Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for about 75-90% of all liver cancer cases. According to the National Comprehensive Cancer Network (NCCN) guidelines, Folinic acid-Fluorouracil-Irinotecan-Oxaliplatin (FOLFIRINOX) is considered a Category 2B option after sorafenib and other therapies for treatment of unresectable HCC, while Gemcitabine-Paclitaxel is considered a Category 3 option. There are multiple clinical trials comparing FOLFIRINOX to Gemcitabine-Paclitaxel including a recent meta-analysis in the journal Hepatology. However, there are limited real-world studies. This abstract compared the overall survival of patients with unresectable HCC treated with FOLFIRINOX or gemcitabine-paclitaxel using real-world data.

**Methods:**A retrospective cohort study was conducted using the TriNetX database, which contained data from 50 million patients from 77 healthcare organizations. Patients 18 years of age and older with unresectable HCC diagnosed between January 1, 2000 and November 19, 2023 were divided into two cohorts: FOLFIRINOX and gemcitabine-paclitaxel. Baseline characteristics, such as age, race, lab findings, comorbidities, and cancer stage, were matched to ensure comparability. Kaplan-Meier curves and paired log-rank tests were used to compare OS between the two cohorts.

**Results:** The median overall survival (OS) was 12.6 months for patients treated with FOLFIRINOX and 9.8 months for patients treated with gemcitabine-paclitaxel. The log-rank test suggested a statistically significant difference in OS between the two groups ($p = 0.020865$), but the risk ratio (0.966) was not significantly different (95% CI: 0.842-1.109).

**Conclusion:** This real-world study provides evidence that FOLFIRINOX does not have statistically significant reduction in mortality compared to gemcitabine-paclitaxel in patients with unresectable HCC. For practitioners considering FOLFIRINOX therapy, there may not be strong justification for tolerating worse side effects compared to gemcitabine-paclitaxel. Further studies are needed to confirm these findings and to identify the optimal chemotherapy regimen for this patient population.

**Figure:**

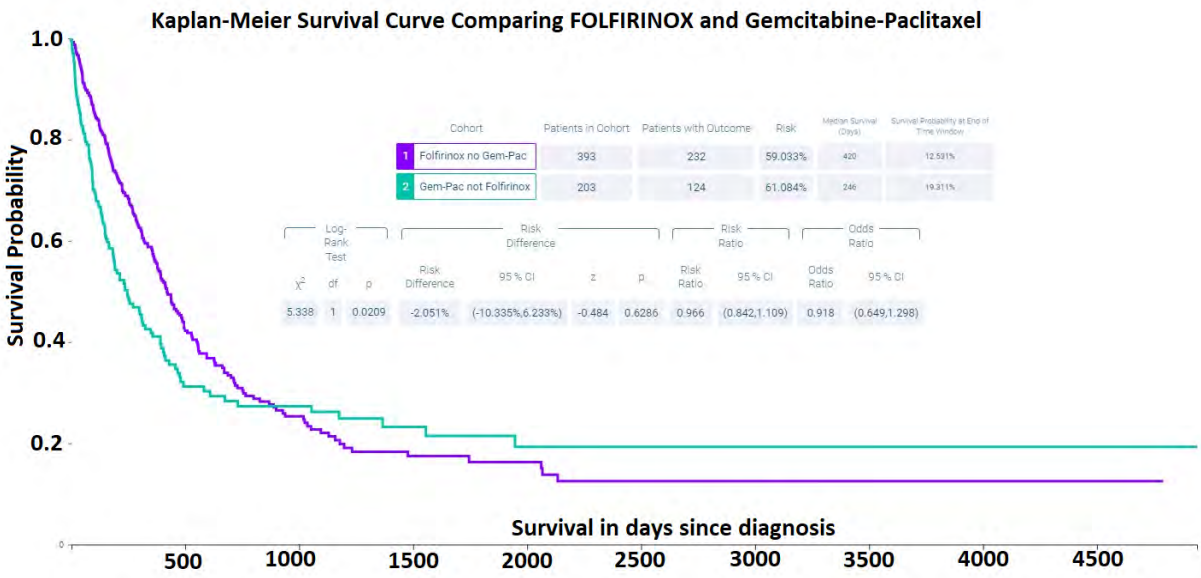


Figure 1: Kaplan-Meier survival curve and summary data comparing FOLFIRINOX and Gemcitabine-Paclitaxel

# PO5-06-YI
# Being part of surveillance programme alone improves early-stage hepatocellular carcinoma detection

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**Background and aims:** Hepatocellular carcinoma (HCC) surveillance increases early-stage detection, allowing curative treatment, and improved survival. Regular surveillance of at-risk individuals using ultrasound (US) with/without alpha-fetoprotein (AFP) 6-monthly for early detection of HCC is recommended. It is unclear if any modifiable factors influence early detection among those who undergo primary surveillance. It is the aim of this study to investigate the impact of these factors on early detection in this patient population.

**Method:** A retrospective analysis of prospectively collected data on patients presented or referred to Nottingham University Hospitals NHS Trust with HCC was undertaken. All patients with a new diagnosis of HCC between 01 January 2019 and 31 December 2022 were included. Those with previous history of HCC presenting with recurrence were excluded. All pre-treatment imaging was independently reviewed by one of three Hepato-Pancreato-Biliary radiologists to assess tumour burden (M. S., A.G., C.C). A liver lesion was deemed HCC if it fulfilled the LR-5 LI -RADS criteria or was histologically confirmed. Early-stage HCC was defined, based on tumour burden, as one tumour less than 50mm or up to 3 tumours all less than 30 mm in maximum diameter. Rate of adherence to surveillance was expressed as proportion of the number of 6-monthly surveillance US performed relative to the total number of scans the patient should have undergone over the preceding 5 years, or since the diagnosis of cirrhosis if it was within the preceding 5 years (longitudinal adherence rate).

**Results:** Of the 224 patients reviewed during the study period, 175 had a new diagnosis of HCC (study cohort). The median age at diagnosis was 71 years (IQR 64-76); 78% (n-136) were males; median BMI was 29.3 (IQR 26.2-33.0); 94% (n = 165) were Caucasians and the most common aetiology of was metabolic dysfunction-associated steatotic liver disease (58%, n = 102). Of the study cohort, a third (n = 64, 37%) presented through primary surveillance (surveillance group); the rest were diagnosed 'incidentally' while being investigated for an unrelated indication (incidental group) (table 1).Age at presentation [p = 0.003; OR 0.937 (95%CI 0.899-0.978)] and being on HCC surveillance [p <0.001, OR 5.867 (95%CI 2.533-13.586)] were the only independent factors associated with early detection. (table 2). Of the surveillance group (n = 64), none of the factors tested, including adherence to surveillance (longitudinal adherence rate), were associated with early HCC detection. (table 3)

**Conclusion:** Being part of primary surveillance, irrespective of adherence rate, is associated with early HCC detection. Given the potential benefits of this, as many patients as possible should be enrolled into primary surveillance programme, even if adherence to recommended frequency is not followed rigorously.

## Figure:

Table 1: Demographic and clinical characteristics of all patients included in the study (n=175), those who were diagnosed to have HCC through surveillance programme (n=64) and those found to have HCC incidentally (n=111).

| | | All (n=175) | Surveillance (n=64) median (IQR) or number (%) | Incidental (n=111) median (IQR) or number (%) | Univariate p value |
|---|---|---|---|---|---|
| **Age at diagnosis (years)** | | 71 (64 – 76) | 70 (65 – 74) | 72 (65 – 77) | 0.13 |
| **Male sex** | | 136 (78%) | 47 (73%) | 89 (51%) | 0.30 |
| **BMI** | | 29.3 (26.2 – 33.0) | 29.8 (25.9 – 33.4) | 29.1 (26.4 – 32.9) | 0.82 |
| **Ethnicity** | **Caucasians** | 165 (94%) | 57 (89%) | 108 (97%) | **0.02** |
| **Indices of Multiple Deprivation Decile** | | 5 (2 – 8) | 5 (2 – 8) | 5 (2 – 8) | 0.87 |
| **Aetiology** | **ArLD** | 32 (18%) | 16 (25%) | 16 (14%) | **<0.00001** |
| | **NAFLD** | 102 (58%) | 31 (48%) | 71 (64%) | |
| | **HCV/HBV** | 19 (11%) | 16 (25%) | 3 (3%) | |
| | **Others** | 22 (13%) | 1 (2%) | 21 (16%) | |
| **Smoking** | **Current** | 25 (14%) | 11 (17%) | 14 (11%) | 0.35 |
| | **Ex** | 89 (51%) | 28 (44%) | 61 (55%) | |
| | **Never** | 61 (35%) | 25 (39%) | 36 (32%) | |
| **Cirrhosis** | | 138 (79%) | 64 (100%) | 74 (67%) | **<0.00001** |
| **CSPH** | | 81 (46%) | 43 (67%) | 38 (34%) | **0.00003** |
| **MELD(3.0) Score** | | 8 (7 – 11) | 9 (7 – 11) | 7 (7 – 10) | **0.03** |
| **UKELD Score** | | 48 (46 – 50) | 48 (47 – 50) | 48 (46 – 50) | 0.29 |

The data is presented as median (interquartile range) or number (percentage). **The p-values that reached statistical significance of <0.05 are indicated in bold**.
**Abbreviations**: ArLD alcohol-related liver disease; BMI body mass index; CSPH clinically significant portal hypertension; HBV hepatitis B; HCV hepatitis C; MELD model for end-stage liver disease score; NAFLD non-alcoholic fatty liver disease; UKELD United Kingdom model for end-stage liver disease score.

**Table 2:** Univariate and multivariate analysis of demographic and clinical characteristics of patients with early stage HCC (n=88) and non-early stage HCC (n=87) of the entire study cohort

| | | Early Stage Detection (n=88) median (IQR) or number (%) | Non-early Stage Detection (n=87) median (IQR) or number (%) | Univariate p value | OR (95%CI) | P value |
|---|---|---|---|---|---|---|
| **Age at diagnosis (years)** | | 69 (62 – 74) | 73 (66 – 77) | 0.001* | 0.937 (0.899 – 0.978) | **0.003** |
| **Male sex** | | 63 (68%) | 73 (93%) | 0.05* | 0.460 (0.187 – 1.131) | 0.09 |
| **BMI** | | 30.1 (27.0 – 34.8) | 28.6 (26.0 – 32.6) | 0.28 | | |
| **Ethnicity** | **Caucasians** | 81 (92%) | 84 (97%) | 0.20 | | |
| **Indices of Multiple Deprivation Decile** | | 5.5 (3 – 8) | 5 (2 – 8) | 0.43 | | |
| **Aetiology** | **ArLD** | 17 (19%) | 15 (17%) | 0.01* | 1.372 (0.535 – 3.520) | 0.51 |
| | **NAFLD** | 47 (53%) | 55 (63%) | | | |
| | **HCV/HBV** | 16 (18%) | 3 (3%) | | | |
| | **Others** | 8 (9%) | 14 (16%) | | | |
| **Smoking** | **Current** | 15 (17%) | 10 (11%) | 0.42 | | |
| | **Ex** | 41 (47%) | 48 (55%) | | | |
| | **Never** | 32 (36%) | 29 (33%) | | | |
| **Cirrhosis** | | 77 (88%) | 61 (70%) | 0.004* | 1.856 (0.668 – 5.159) | 0.24 |
| **CSPH** | | 47 (53%) | 34 (39%) | 0.06* | 0.727 (0.324 – 1.634) | 0.78 |
| **Presentation** | **Surveillance** | 50 (57%) | 14 (16.1%) | <0.00001* | 5.867 (2.533 – 13.586) | **<0.001** |
| **MELD(2016) Score** | | 8 (7 – 11) | 8 (7 – 11) | 0.31 | | |
| **UKELD Score** | | 48 (46 – 50) | 48 (46 – 50) | 0.75 | | |

The data is presented as median (interquartile range) or number (percentage). *indicates the parameters that were included in the multivariate analysis. **The Bonferroni-corrected level of significance in this analysis was p <0.0083 and is indicated in bold**.
**Abbreviations**: ArLD alcohol-related liver disease; BMI body mass index; CSPH clinically significant portal hypertension; HBV hepatitis B; HCV hepatitis C; MELD model for end-stage liver disease score; NAFLD non-alcoholic fatty liver disease; UKELD United Kingdom model for end-stage liver disease score.

**Table 3:** Univariate and multivariate analysis of demographic and clinical characteristics of patients with early stage HCC (n=50) and non-early stage HCC (n=14) of those diagnosed through primary surveillance alone (Surveillance cohort)

| | | Early Stage Detection (n=50) median (IQR) or number (%) | Non-early Stage Detection (n=14) median (IQR) or number (%) | Univariate p value | OR (95%CI) | P value |
|---|---|---|---|---|---|---|
| **Age at diagnosis (years)** | | 68 (63 – 73) | 72 (67 – 76) | 0.11 | | |
| **Male sex** | | 34 (68%) | 13 (93%) | 0.06* | 0.202 (0.022 – 1.857) | 0.16 |
| **BMI** | | 29.6 (25.9 – 35.3) | 32.1 (25.9 – 32.8) | 0.58 | | |
| **Ethnicity** | **Caucasians** | 45 (90%) | 12 (86%) | 0.65 | | |
| **Indices of Multiple Deprivation Decile** | | 6 (3 – 8) | 4 (2 – 7) | 0.16 | | |
| **Aetiology** | **ArLD** | 13 (26%) | 3 (21%) | 0.17 | | |
| | **NAFLD** | 21 (42%) | 10 (71%) | | | |
| | **HCV/HBV** | 15 (30%) | 1 (7%) | | | |
| | **Others** | 1 (2%) | 0 (0%) | | | |
| **Smoking** | **Current** | 10 (20%) | 1 (7%) | 0.53 | | |
| | **Ex** | 21 (42%) | 7 (50%) | | | |
| | **Never** | 19 (38%) | 6 (43%) | | | |
| **CSPH** | | 32 (64%) | 11 (79%) | 0.30 | | |
| **Duration of primary surveillance** | | 50 (28 – 104) | 34 (18 – 56) | 0.13 | | |
| **Adherence to surveillance (%)** | | 80 (67 – 100) | 67 (55 – 82) | **0.04*** | 1.024 (0.991 – 1.057) | 0.15 |
| **MELD(2016) Score** | | 9 (7 – 11) | 11 (9 – 13) | **0.03*** | 0.851 (0.686 – 1.055) | 0.14 |
| **UKELD Score** | | 48 (46 – 50) | 48 (47 – 54) | 0.18 | | |

The data is presented as median (interquartile range) or number (percentage). *indicates the parameters that were included in the multivariate analysis. The Bonferroni-corrected level of significance in this analysis was p <0.016.
**Abbreviations**: ArLD alcohol-related liver disease; BMI body mass index; CSPH clinically significant portal hypertension; HBV hepatitis B; HCV hepatitis C; MELD model for end-stage liver disease score; NAFLD non-alcoholic fatty liver disease; UKELD United Kingdom model for end-stage liver disease score.

# PO5-09
# Novel ctDNA technology for liver cancer detection by immunoprecipitation of tumor associated ctDNA fragments and analysis by qPCR

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**Background and aims:** CTCF transcription factor binding is altered in cancer including both gain and loss of CTCF occupancy. We hypothesised that chromatin immunoprecipitation (ChIP) of CTCF-DNA (cfCTCF-DNA) nucleoproteins from plasma might isolate cancer associated gain of occupancy CTCF binding site sequences from all non-cancer derived plasma cfDNA of the same sequences which would be nucleosome covered and removed by ChIP. Current ctDNA methods are based on DNA fragment sequence and size. We aimed to obviate DNA library preparation, Next Generation Sequencing and bioinformatics by means of a CTCF-ChIP/PCR analysis to facilitate a low cost, rapid, automatable, high throughput ctDNA technology. We now report the results of this analysis on cohorts of patients diagnosed with hepatic and bile duct cancer.

**Method:** We isolated plasma cfCTCF-DNA nucleoproteins by ChIP. We performed plasma CTCF ChIP-Seq experiments to identify cancer associated CTCF gain of occupancy cancer sequences by analysis of plasma samples taken from acute myeloid leukaemia (AML) patients as well as patients with inflammatory conditions and healthy volunteers. Suitable gain of occupancy sequences were selected as characterised CTCF binding site sequences that were present in the CTCF ChIP isolates of AML patients but absent from isolates from patients without cancer. We then developed qPCR assays for selected CTCF gain of occupancy biomarker sequences. We confirmed that CTCF gain of occupancy biomarkers would identify patients with AML, liver or bile duct cancer in an initial small test experiment (n = 31, 5 and 5 respectively). We subsequently conducted a study in a larger cohort of patients.

**Results:** In our initial experiments, a simple cutoff where a qPCR result exceeding the cutoff was positive, a single qPCR assay detected 19/31 AML cases (61%) with 1 false positive result among 50 control samples (98% specificity). On the same basis a different single qPCR detected 3/5 hepatic and 4/5 bile duct cancer cases tested using a cutoff of 3.5 with 4 false positive results (92% specificity) (Table). We now report the results of investigations of larger cohorts of patients with hepatic and bile duct cancers.

**Conclusion:** Combining the isolation of plasma cfCTCF-DNA nucleoproteins with PCR analysis of cancer associated CTCF gain of occupancy binding site sequences is a novel ctDNA analysis technology that may provide the basis of rapid, low cost, automated liquid biopsy methods.
Further discovery experiments using liver and bile cancer models, rather than an AML model, and further clinical studies are required.

# PO5-10
# Non-viral etiology with low baseline neutrophil to lymphocyte ratio (NLR) and liver disease severity could predict clinical outcome in Atezolizumab-Bevacizumab treated Caucasian cirrhotic patients with advanced hepatocellular carcinoma

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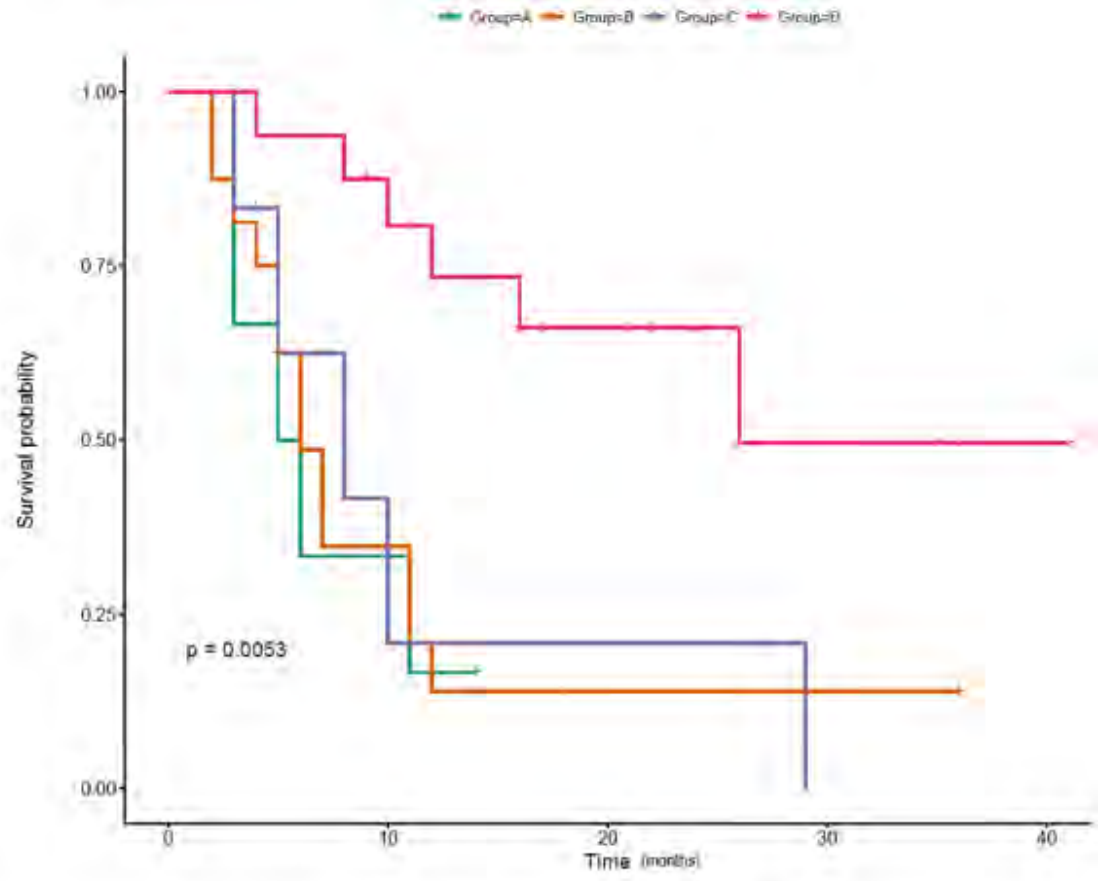
**Background and aims:** Non-viral etiology of chronic liver disease has not been related with survival benefit in patients with advanced HCC treated with Atezo-Bev. Moreover, a high NLR has been related with low response rates and worse clinical outcomes whereas data regarding the histological evaluation of these patients are limited. The aim of our study was to evaluate the survival of Caucasian cirrhotic patients with advanced HCC treated with Atezo-Bev according to viral/non-viral etiology of chronic liver disease as well as baseline NLR and proliferative/non-proliferative subgroup histological HCC status.

**Method:** Forty-four cirrhotic patients with histologically documented advanced HCC were included (35 males, mean age = 66.6, median MELD/Na = 8, 39 CPT-A, 22 ALBI-I, 12 with varices, 18 with MVI, 17 with EHD, 22 with viral and 22 with non-viral etiology). Twenty-three HCCs were classified by morphomolecular analysis as proliferative (PR) and 21 as non-proliferative disease (N-PR), according to the classification by Nault JC et al, J Hepatol 2018. Baseline NLR was evaluated in all patients at the first day of Atezo-Bev treatment and a ratio above 4 was considered as high. Four groups were constructed [A: V/NLR-H (n = 6), B: V/NLR-L (n = 16), C: NV/NLR-H (n = 6), D: NV/NLR-L (n = 16)], according to etiology and NLR status. Patients with complete or partial response according to mRECIST criteria were categorized as having objective response (OR).

**Results:** PR disease was observed in 13/23 (56.5%) patients of viral and 10/23 (43.5%) patients of non-viral etiology whereas N-PR disease was observed in 9/21 (42.8%) patients of viral and 12/21 (57.2%) of non-viral etiology. Totally OR was observed in 9/44 (20.45%) patients (0/3/2/4 from group A/B/C/D respectively) and was significantly related with overall survival (OS, p = 0.009). All four groups were comparable for all the baseline parameters evaluated except for age (p = 0.018), MELD/Na (p = 0.007), presence of varices (p = 0.019) and MVI (p = 0.001). Median OS was 5.5m for group A, 6m for B, 8m for C and 26m for D. We observed a significant correlation with the best OS in patients with low baseline NRL of non-viral etiology (p = 0.0053, figure) compared to the other groups. In the multivariate analysis, considering age, MELD/Na, varices and MVI, only group D patients (HR = 0.18, 0.04-0.71, p = 0.014) and MELD/Na (HR = 1.21, 1.02-1.43, p = 0.027) were significantly correlated with survival benefit.

**Conclusion:** Proliferative and non-proliferative morphomolecular subgroup HCCs were equally observed among Caucasian cirrhotic patients with advanced HCC of viral or non-viral liver disease. Non-viral etiology per se should not be considered as a negative predictor of clinical outcome in Caucasian cirrhotic patients with advanced HCC treated with Atezo-Bev, as the subgroup of them who present low baseline NLR values seem to benefit the most from treatment.

**Figure:**




| | Hazard Ratio | 95% CI for HR | p-value |
|---|---|---|---|
| Group B vs Group A | 0.57 | 0.18 to 1.81 | 0.3 |
| Group C vs Group A | 0.65 | 0.13 to 3.24 | 0.6 |
| Group D vs Group A | 0.18 | 0.04 to 0.71 | 0.014 |
| Varices | 1.10 | 0.46 to 2.65 | 0.8 |
| MVI | 0.91 | 0.33 to 2.52 | 0.9 |
| MELD-Na | 1.21 | 1.02 to 1.43 | 0.027 |
| Age | 1.01 | 0.97 to 1.06 | 0.6 |

# PO5-14-YI
# Adverse events (AEs) as potential predictive factors of activity in patients with advanced hepatocellular carcinoma (HCC) treated with atezolizumab plus bevacizumab (AB)

Mara Persano[1], Margherita Rimini[2], Toshifumi Tada[3], Goki Suda[4], Shimose Shigeo[5], Masatoshi Kudo[6], Changhoon Yoo[7], Jaekyung Cheon[8], Fabian Finkelmeier[9], Ho Yeong Lim[10], José Presa[11], Gianluca Masi[12], Francesca Bergamo[13], Massimo Iavarone[14], Giuseppe Cabibbo[15], Francesco Giuseppe Foschi[16], Fabio Piscaglia[17], Silvia Foti[2], Silvia Camera[18], Noemi Cornara[2], Francesco Vitiello[2], Elisabeth Amadeo[2], Federico Rossari[2], Stefano Cascinu[2], Mario Scartozzi[1], Andrea Casadei Gardini[2]

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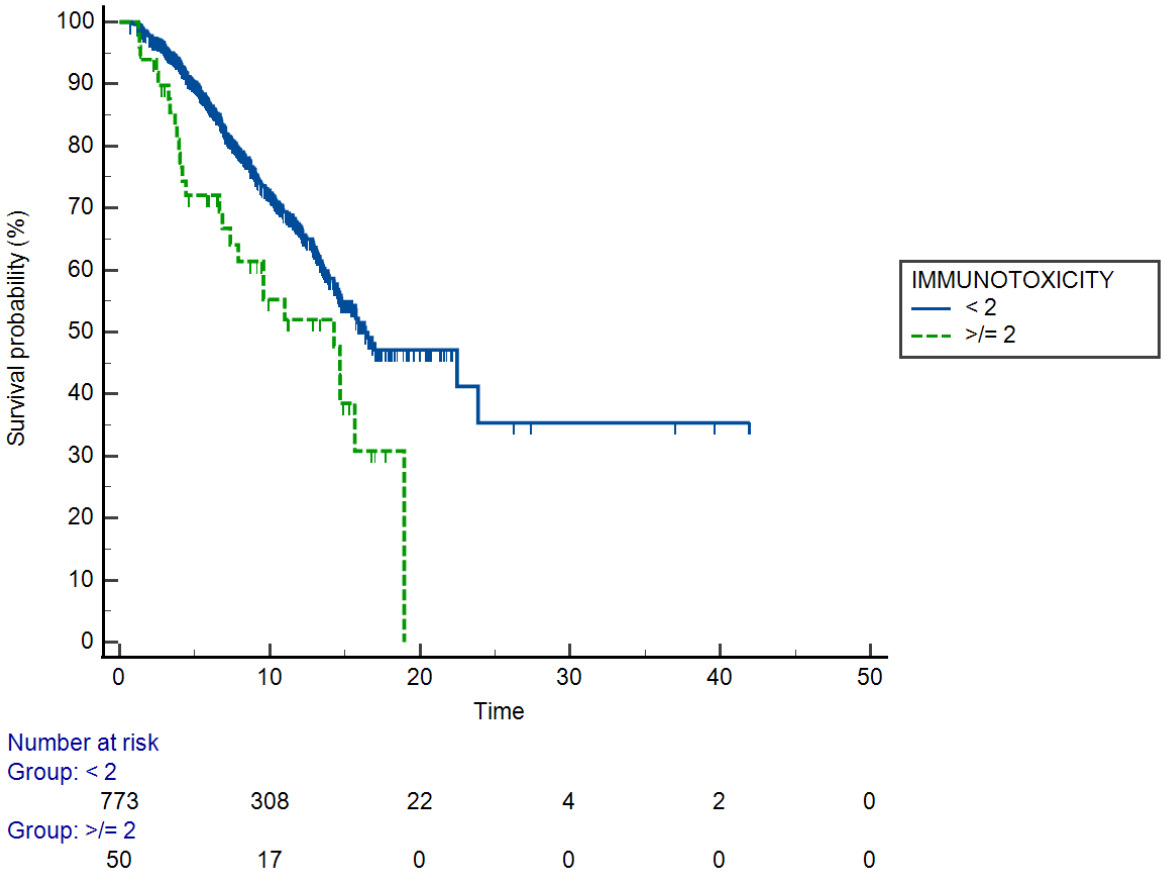
**Background and aims:** This retrospective multicenter real-world study aims to investigate the potential prognostic value of AEs in HCC patients treated with AB in first-line setting.

**Method:** The study's population consisted of 823 HCC patients from five countries (Italy, Germany, Portugal, Japan, and the Republic of Korea) treated with AB between October 2018 and April 2022.

**Results:** Median overall survival (OS) was 15.9 months and median progression-free survival (PFS) was 7.6 months. 73.3% of patients presented at least one AE during the study period. The most common AEs were proteinuria (29.6%), arterial hypertension (27.2%), and fatigue (26.0%). 17.3% of the AEs were grade (G) 3. One death due to bleeding was reported. The multivariate analysis confirmed the appearance of decreased appetite G <2 versus G ≥2 [hazard ratio (HR): 0.60; p <0.01] and immunotoxicity G <2 versus G ≥2 (HR: 0.70; p = 0.04) as independent prognostic factors for OS, and the appearance of decreased appetite G <2 versus G ≥2 (HR: 0.73; p = 0.01), diarrhea of any G versus no diarrhea (HR: 0.57; p = 0.01), fatigue of any G versus no fatigue (HR: 0.82; p <0.01), arterial hypertension G <2 versus G ≥2 (HR: 0.68; p <0.01), and proteinuria of any G versus no proteinuria (HR: 0.79; p = 0.03) as independent prognostic factors for PFS. The objective response rate (ORR) was 27.3%: complete responses (CR) were 35 (4.2%), partial response 190 (23.1%), stable disease 428 (52.0%), and progressive disease 170 (20.7%). The appearance of hypothyroidism of any G (odds ratio: 0.52; p = 0.04) and immunotoxicity of any G (odds ratio: 0.54; p <0.01) were correlated with higher ORR, while the absence of fatigue of any G (odds ratio: 3.90; p = 0.02) and decreased appetite of any G (odds ratio: 9.71; p = 0.02) were correlated with more CR.

**Conclusion:** As also demonstrated for other therapies, also for the combination of AB there is a correlation between the occurrence of AEs and HCC patient outcomes.

**Figure:**


100
90
80
70
60
50
40
30
20
10
0
Survival probability (%)
0
10
20
30
40
50
Time
IMMUNOTOXICITY
< 2
>/= 2
Number at risk
Group: < 2
773 308 22 4 2 0
Group: >/= 2
50 17 0 0 0 0

# PO5-15-YI
# Impact of ISO score on oncological outcomes and survival in liver transplant candidates with hepatocellular carcinoma

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**Background and aims:** Liver transplantation (LT) is the most efficacious curative treatment for hepatocellular carcinoma (HCC). Waiting list (WL) prioritization criteria determine patient outcomes. Since 2015, the Italian Score for Organ Allocation (ISO) has been used in Italy to prioritize patients on the WL, introducing a specific policy for HCC. In this study, we aimed to assess the impact of ISO-score on WL dropout, HCC recurrence, and patient survival in LT candidates with HCC.

**Method:** This retrospective study included all patients with HCC listed for LT (n = 378) at our institute between January 2013 and December 2020. We considered 2 groups: patients prioritized before (group A, 2013-2015) and after (group B, 2016-2020) ISO score implementation. We used cumulative incidence functions within a competing risk time-to-event analysis to assess and compare, between the 2 groups, the probabilities of LT, WL death and dropout. Uni- and multivariable logistic regression models were also used to identify potential risk factors associated with WL mortality.

**Results:** Group B patients were older than group A patients (p = 0.01), presenting more grading 3 HCC in the explanted liver (group A: 8.3%, group B: 16.8%; p = 0-04). After the implementation of ISO score, a higher rate of HCC MASLD-related (group A: 7%, group B: 11.7%; p = 0-03) have been registered.During a median follow-up of 49 months, 289 HCC patients underwent LT, 4 patients (13%) died before LT, and 71 patients (18%) were removed from WL for HCC progression. According to the competing risk multivariate analysis, WL dropout rate was similar in the two groups (p = 0.07). Downstaging treatments (p = 0.0001) and AFP levels (p = 0.0001) were identified as risk factors for WL dropout. HCC recurrence after LT rate was significantly lower in group B patients than group A (p = 0.03) (Fig.1), also confirmed at multivariate analysis (p = 0.04). Group B patients showed higher overall survival (OS) than group A patients (p = 0.04), without difference in no-HCC related deaths between the two groups (p = 0.049) (Fig.2).

**Conclusion:** After the introduction of ISO score, a lower HCC recurrence after LT has been observed as well as an improved overall survival after LT. These findings strongly suggest that the implementation of the ISO score has enhanced the allocation process for HCC patients, resulting in a substantial increased transplant benefit.

**Figures:**

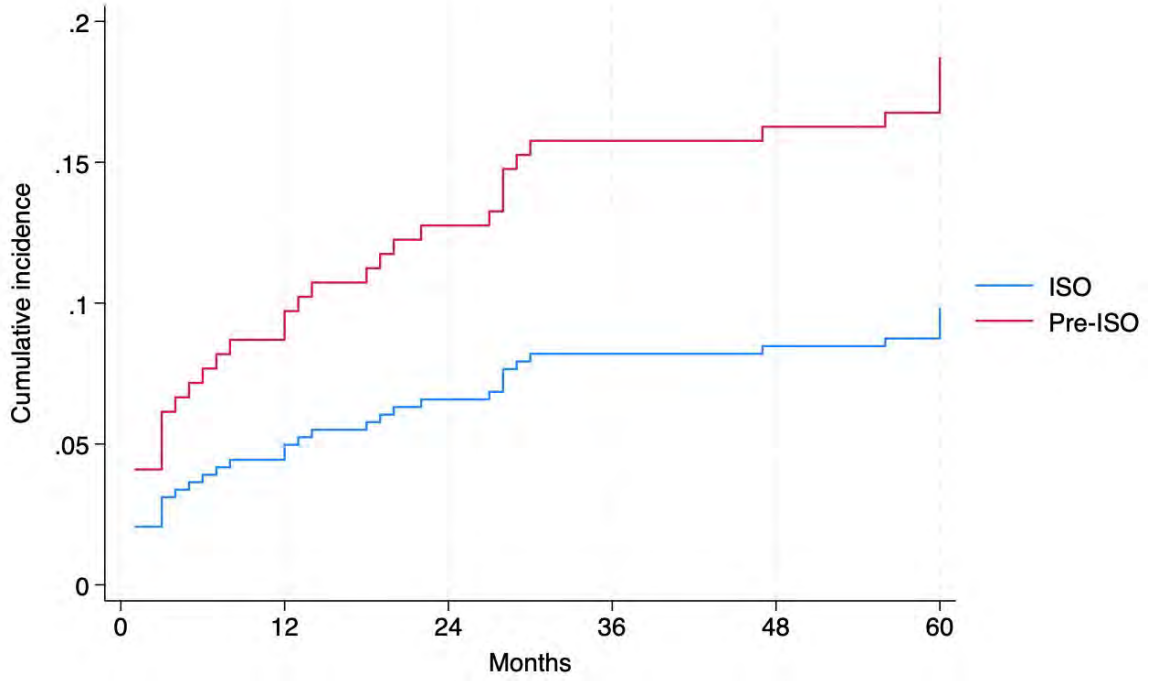


**Fig. 1 HCC recurrence rate after LT**

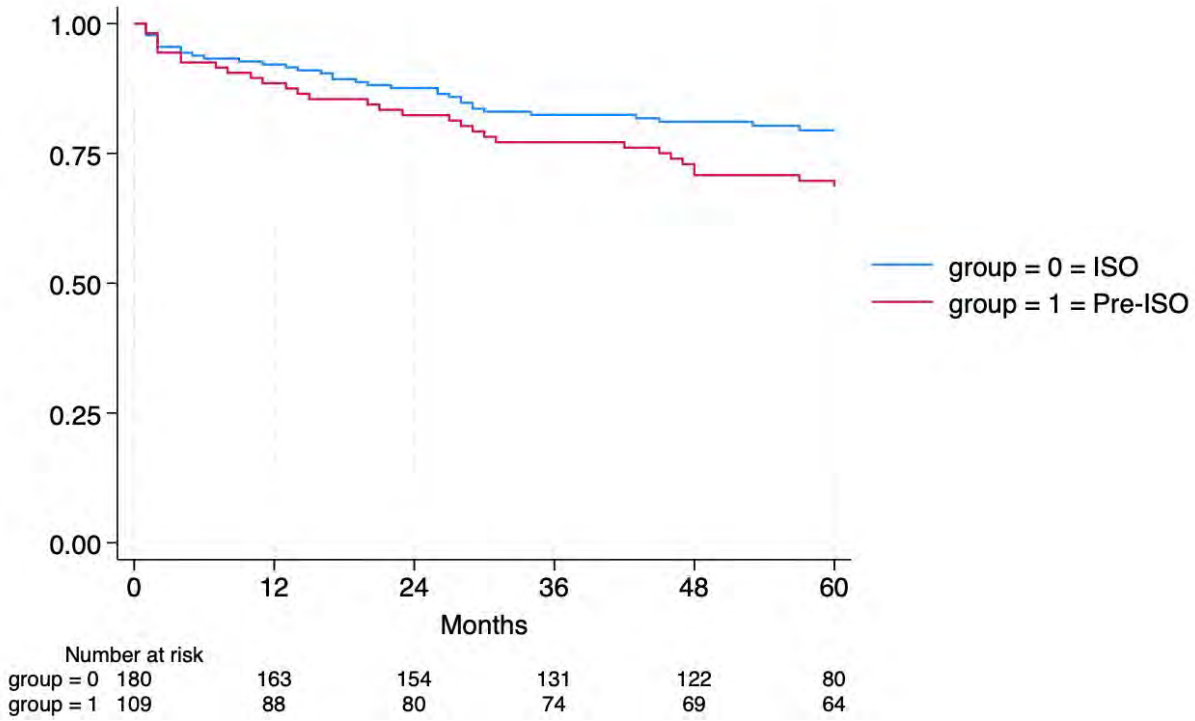


**Fig. 2 OS of HCC-patients undergoing LT**

# PO5-16-YI
# Immunotherapy-tyrosine kinase inhibitor sequences for patients with advanced hepatocellular carcinoma: a single center, retrospective, comparative study

Angelo Pirozzi[1 2], Giulia Tesini[1 2], Valentina Zanuso[1 2], Rita Balsano[1 2], Tiziana Pressiani[1], silvia bozzarelli[1], Lorenza Rimassa[1 2]

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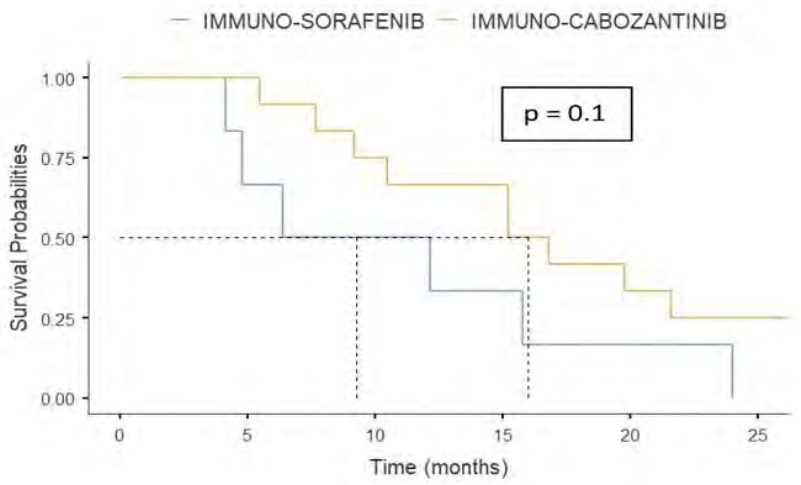
**Background and aims:** The IMbrave150 and HIMALAYA phase 3 trials established atezolizumab-bevacizumab and durvalumab-tremelimumab as new first-line standards of care for patients with advanced or unsuitable for surgery/locoregional therapy HCC. The choice of the most effective treatment sequence at the time of progression to immunotherapy regimens is an unsolved issue, with tyrosine kinase inhibitors (TKIs) being the most common options. Our aim is to describe the clinical outcomes of patients treated with TKIs after progression to first-line immunotherapy treatments.

**Method:** This is a single center, retrospective, comparative study. The primary outcome is progression free survival (PFS) calculated from the start of first-line immunotherapy to progression to second line TKIs. Secondary outcome is PFS difference between patients receiving sorafenib (immunotherapy-sorafenib sequence) and cabozantinib (immunotherapy-cabozantinib sequence) in the second-line setting assessed by Kaplan-Meier and log-rank tests.

**Results:** Between October 2020 and November 2023, 21 patients with ECOG PS 0-1, Child Pugh A liver function, and BCLC-B (10%) or BCLC-C (90%) HCC received either cabozantinib or sorafenib upon progression to immunotherapy. First-line treatments included atezolizumab-bevacizumab (33%), durvalumab-tremelimumab (14%), durvalumab (14%), atezolizumab-cabozantinib (6%), or other regimens (33%). 14 patients (67%) received the immunotherapy-cabozantinib sequence, and 7 patients (33%) the immunotherapy-sorafenib sequence. At a median follow-up of 22 months, median PFS (mPFS) for the overall population was 15 months (95% CI, 9-24). mPFS was 16 months (95% CI, 10.47-NR) for the immunotherapy-cabozantinib sequence and 9 months (95% CI, 4.80-NR) for the immunotherapy-sorafenib sequence. mPFS was numerically longer for the immunotherapy-cabozantinib sequence but the difference with the immunotherapy-sorafenib sequence (delta = 6.7 months) was not statistically significant (p = 0.1) [Figure].

**Conclusion:** Immunotherapy-TKI sequence is a valid option for patients with unresectable/advanced HCC compared to the TKI monotherapy era. The immunotherapy-cabozantinib sequence showed a numerically longer mPFS compared to the immunotherapy-sorafenib sequence but the difference between the two sequences was not statistically significant, likely due to the small sample size and heterogeneity of first-line treatments. Nonetheless, the difference between the two groups was remarkably high (6.7 months), thus deserving further evaluation in a larger prospective analysis to minimize potential selection biases.

**Figure:**


IMMUNO-SORAFENIB
IMMUNO-CABOZANTINIB
p = 0.1
Survival Probabilities
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
25
Time (months)

# PO6-03
# Lenvatinib (L) versus sorafenib (S) second-line therapy in hepatocellular carcinoma (HCC) patients progressed to atezolizumab plus bevacizumab (AB)

Mara Persano[1], Margherita Rimini[2], Toshifumi Tada[3], Goki Suda[4], Shimose Shigeo[5], Masatoshi Kudo[6], Changhoon Yoo[7], Jaekyung Cheon[8], Fabian Finkelmeier[9], Ho Yeong Lim[10], José Presa[11], Gianluca Masi[12], Francesca Bergamo[13], Massimo Iavarone[14], Giuseppe Cabibbo[15], Francesco Giuseppe Foschi[16], Fabio Piscaglia[17], Silvia Foti[2], Silvia Camera[18], Noemi Cornara[2], Francesco Vitiello[2], Elisabeth Amadeo[2], Federico Rossari[2], Stefano Cascinu[2], Mario Scartozzi[1], Andrea Casadei Gardini[2]

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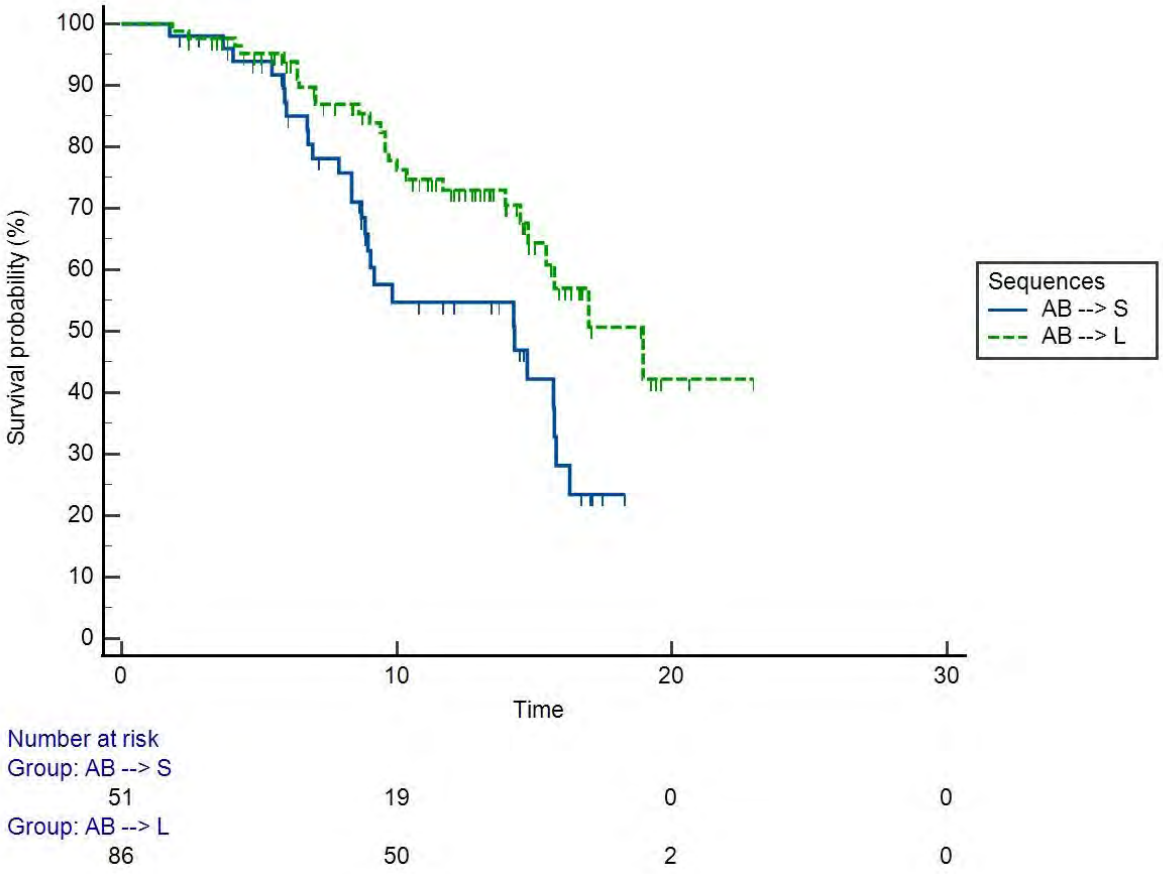
**Background and aims:** This retrospective multicenter real-world study aims to compare outcomes reached by L and S second-line therapy in HCC patients treated with first-line AB.

**Method:** The overall cohort included 891 HCC patients from five countries (Italy, Germany, Portugal, Japan, and the Republic of Korea) treated with AB in first-line setting between October 2018 and April 2022. A total of 472 (53.0%) patients had progressive disease after first-line therapy, of which 243 (51.5%) received a second-line treatment. Data from 137 patients were available for the analysis: 51 (37.2%) received S and 86 (62.8%) L.

**Results:** L second-line subgroup achieved a median overall survival (mOS) of 18.9 months, significative longer (p = 0.01; HR: 2.24) compared to S subgroup that reached a mOS of 14.3 months. After adjusting for positive clinical covariates at univariate analysis, multivariate analysis highlighted Albumin-Bilirubin (ALBI) 1 grade [p <0.01; hazard ratio (HR): 5.23] and L second-line therapy (p = 0.01; HR: 2.18) as positive prognostic factor for OS. Forest plot highlighted a positive trend in terms of OS in favor of patients treated with L second-line regardless of baseline characteristics before first-line therapy. In particular, L second-line subgroup had a better OS compared to S second-line subgroup in male patients, aged ≤70 years, with viral etiology, Barcelona Clinic Liver Cancer C stage, αfetoprotein <400 ng/ml, Child-Pugh A, NLR <3, ALBI 1 grade, performance status ≤1, presence of portal vein thrombosis. Regarding first-line treatment outcomes, L second-line subgroup achieved a median progression-free survival (mPFS) of 3.5 months, while S second-line subgroup reached a mPFS of 4.3 months without any significative difference (p 0.42; HR: 1.15). There was no difference in overall response rate (L 26.1% vs. S 19.8%; p = 0.29) and disease control rate (L 76.8% vs. S 66.4%; p = 0.71) between the two subgroups.

**Conclusion:** L second-line therapy is superior to S in HCC patients progressed to first-line AB.

**Figure:**


Survival probability (%)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
Time
Sequences
AB --> S
AB --> L
Number at risk
Group: AB --> S
51
19
0
0
Group: AB --> L
86
50
2
0

# PO6-04-YI
# Evolution of liver function during immune checkpoint inhibitor treatment for hepatocellular carcinoma

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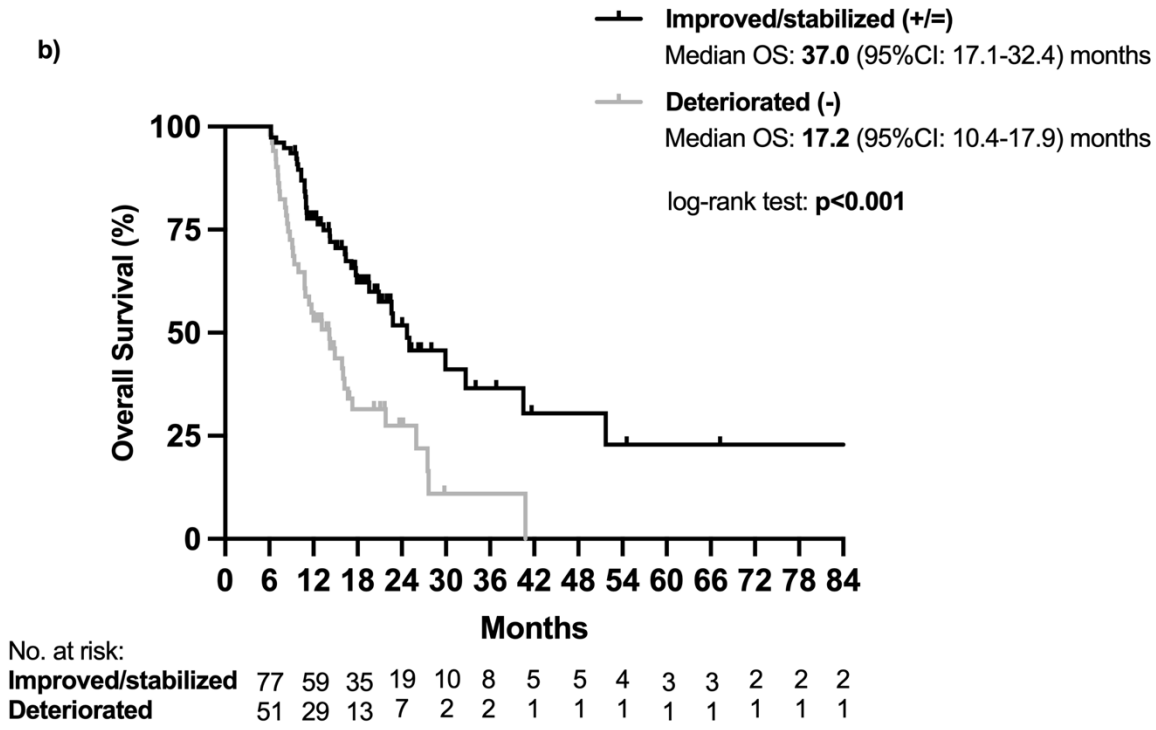
**Background and aims:** Deterioration of liver function is a leading cause of death in patients with advanced hepatocellular carcinoma (HCC). We evaluated the impact of immune checkpoint inhibitor (ICI)-treatment on liver function and outcomes.

**Method:** HCC patients receiving ICIs or sorafenib between 04/2003-08/2023 at two institutions were included. Liver function-as assessed by Child-Pugh score (CPS)-was evaluated at the start of ICI-treatment (baseline, BL) as well as 3 and 6 months thereafter. A ≥1 point change in CPS was defined as deterioration (-) or improvement (+), while equal CPS points were defined as stable ( = ).

**Results:** Overall, 182 ICI-treated patients (66.8 ± 11.8 years; male: n = 145, 80%; cirrhosis: n = 134, 74%) were included. At BL, median CPS was 5 (IQR: 5-6; CPS-A: 146, 80%). After 3 months, liver function improved/stabilized in 100 (55%) and deteriorated in 63 (35%) patients while 19 (10%) patients deceased/had missing follow-up (noFU). Comparable results were observed at 6 months (+/= : n = 79, 43%; -: n = 53, 29%; deceased/noFU: n = 50, 28%). Radiological response was linked to improvement/stabilization of CPS at 6 months (responders vs. non-responders, 73% vs. 51%; p = 0.011). Improvement/stabilization of CPS at 6 months was associated with a better overall survival (OS) following landmark analysis (6 months: +/= : 37.0 (95%CI: 17.1-32.4) vs. -: 17.2 (95%CI: 10.4-17.9) months; p <0.001). In contrast, 54 (34%) and 33 (21%) out of 160 sorafenib patients achieved an improvement/stabilization, while 46 (29%) and 39 (24%) patients deteriorated at 3 and 6 months, respectively. Of 36 ICI-patients with CPS B at BL, improvement/stabilization was observed in 17 (47%) patients while 19 (53%) patients deteriorated/deceased/noFU at 3 months. Comparable results were observed at 6 months (CPS +/= : 14, 39%, -: 11, 31%). In total, 5/36 (14%) patients improved from CPS B to CPS A at both timepoints.

**Conclusion:** Radiological response to ICI-treatment was associated with stabilization or improvement in liver function which correlated with improved survival, even in patients with Child-Pugh class B at baseline.

**Figure: Overall survival according to evolution of liver function in patients with hepatocellular carcinoma treated with immunotherapy.** Landmark analysis at 6 months comparing overall survival (OS) between patients who achieved an improvement/stabilization of liver function at the landmark compared to those with deterioration of liver function.



# PO6-05
# The impact of age gender on the survival of patients diagnosed hepatocellular carcinoma

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**Background and aims:** While age and gender have been identified as significant risk factors for hepatocellular carcinoma (HCC) occurrence, there is conflicting evidence regarding their association with HCC survival. The objective of this study is to investigate the relationship between gender, patient age at diagnosis, and overall survival (OS) in HCC patients.

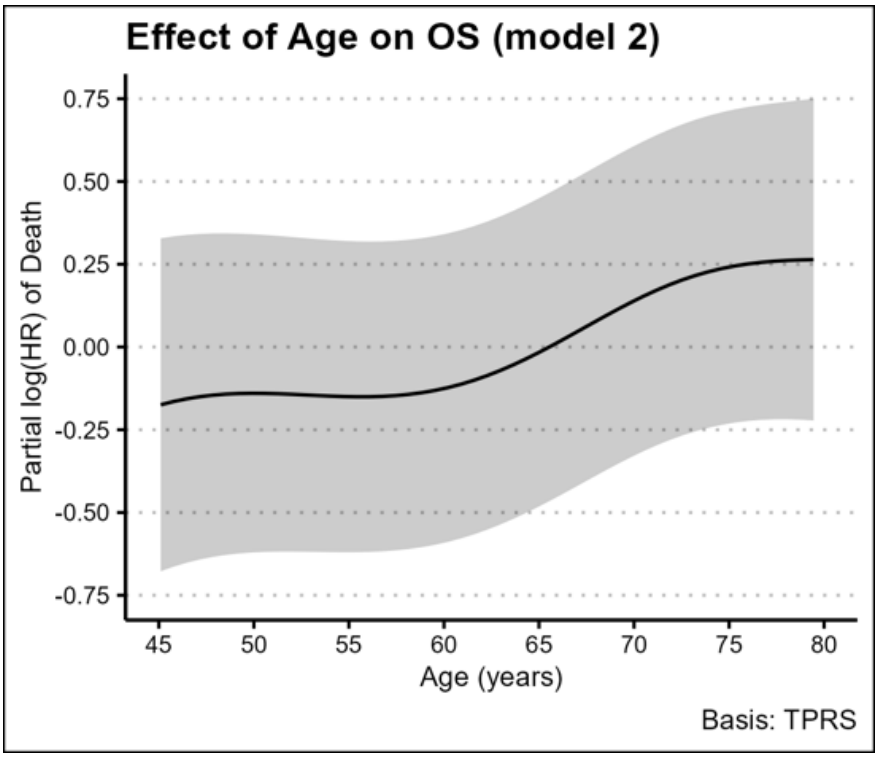
**Method:** Data of two HCC cohorts (HCC cohort of University Hospital of Bern, Switzerland and HCC cohort of the Medical University of Vienna, Austria) were combined and analyzed. Clinical data and laboratory results were reviewed and overall survival was compared by using Kaplan-Meier curves and the log-rank test. Cox regression models with penalized splines were utilized to assess how age at time of diagnosis (as a continuous variable) affects OS. Regression adjustment was used to further explore possible causes of the detected association between age and OS.

**Results:** Among the 1.547 patients diagnosed with hepatocellular carcinoma (HCC), 1.284 (84.1%) were male. Notably, females exhibited a statistically significant older age compared to males (67 years vs. 63 years, $p < 0.001$) and presented with fewer comorbidities (i.e. diabetes, arterial hypertension). The majority of patients were male, were cirrhotic (88%) classified as Child Pugh A (47%) and staged as BCLC 0 and A (44%). Approximately 41% underwent curative therapy (i.e. transplant, resection, ablative therapy).

No significant disparity in the median overall survival (OS) was observed between men and women (18 months versus 16 months; $p = 0.304$). The risk of mortality exhibited an upward trend with increasing age, without a discernible cutoff point, with a more pronounced effect noted in the age range of 60-65 years. Upon further analysis, when controlling for the type of treatment, the impact of age persisted in the same direction but diminished in magnitude and lost statistical significance ($p = 0.171$).

**Conclusion:** This study shows that gender has no impact on OS of HCC patients whereas age is associated with survival, possibly due to its impact on treatment decisions and co-morbidities

**Figure:**


Effect of Age on OS (model 2)
Partial log(HR) of Death
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
45
50
55
60
65
70
75
80
Age (years)
Basis: TPRS

# PO6-06-YI
# Assessment of liver fibrosis with transient elastography in non-alcoholic fatty liver disease patients; a real-life experience from Pakistan

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**Background and aims:** Transient Elastography (TE) is a non-invasive technique for estimating liver fibrosis. There is limited data about the performance of TE in Pakistani patients with non-alcoholic fatty liver disease (NAFLD). NAFLD is a global outbreak, it is pivotal that patients with NAFLD should undergo an assessment for their risk of advanced fibrosis, which enhances the risk of hepatocellular carcinoma (HCC) and other complications of cirrhosis. In the present study, we have evaluated the diagnostic accuracy of TE in identifying different degrees of fibrosis in NAFLD adult patients.

**Method:** A Cross-sectional study was undertaken at the Department of Gastroenterology, Jinnah Postgraduate Medical Centre Karachi, Pakistan. After obtaining ethical approval, all patients above the age of 18 years, with a diagnosis of NAFLD on the basis of abnormal liver function tests (LFTs) and an ultrasound abdomen consistent with fatty liver were included in the study. All patients with hepatitis, hepatic malignancies, hepatobiliary infections, and biliary tract disease were excluded from the study. Fibrosis score was calculated through Elastography as F0-F1 (5.3-7.1 kPa, Normal); F2 (7.5-8.5 kPa, Mild/Grade-I); F3 (9.5-13.0 kPa, Moderate/Grade-II); and F4 (13.1-18.8 kPa, Severe/Grade-III).

**Results:** A total of 162 patients were enrolled in the study, from which 121 (75%) were female 41 (25 %) were male with a mean age of 39.6 ± 9.7 years. Of these, 108 (66.6 %) belonged to the lower socioeconomic class. One hundred and twenty-one (75%) of patients had fatty liver while 41 (25%) had hepatomegaly with fatty changes. TE revealed that 39.5 % (64) had a score of F0- F1, and 37.7 % (61) showed mild grade stiffness depicting fibrosis with a score of F2. Patients with moderate (F3) and severe (F4) grade fibrosis were 17.3 % (28) and 5.6 % (9).

**Conclusion:** Detecting liver fibrosis at its early stages is crucial in preventing its progression to cirrhosis, which is irreversible. Reversal of fibrosis is only possible if it is diagnosed as early as possible and managed with appropriate treatment.

## PO6-07-YI

# Competitive risk analysis of spleen stiffness measurement with a spleen-dedicated module (SSM@100Hz) for predicting de-novo HCC occurrence in cACLD patients: a prospective 5-year follow-up study

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**Background and aims:** Hepatocellular carcinoma (HCC) impact significantly the survival of patients with liver disease and has been revealed that portal hypertension (PH) is a major contributor to its development. Notably, clinically significant portal hypertension (CSPH), as identified by HVPG, can predict the occurrence of HCC. To explore the potential of Spleen stiffness measurement (SSM) as a predictor of HCC occurrence, particularly with the use of a new spleen-dedicated module (SSM@100hz), we conducted a prospective study. Our investigation aimed to determine whether SSM@100hz could identify the HCC occurrence in individuals with compensated advanced chronic liver disease (cACLD).

**Methods**: We conducted a prospective study on patients who underwent paired laboratory exams, hepatic venous pressure gradient measurement (HVPG), liver stiffness measurement (LSM), and SSM@100hz. The patients were followed as per the current international guidelines for HCC screening, and we assessed the occurrence of HCC or other complications related to liver disease. We used Competing-risks regression analysis to account for liver transplantation or death as competitive events.

**Results:** We enrolled 69 patients with a median follow-up of 68 months until HCC or a competitive event occurrence. Table 1 reported the characteristics of patients included with HCC occurrence at competitive univariate statistical analysis was associated with a higher body mass index (BMI), SSM median value (SSM@100hz), HVPG, metabolic etiology, and combined Baveno VII single cut-off rule-in criteria (by SSM@100hz). HCC occurrence at multivariate analysis based on SSM median values (SSM@100hz) was associated with SSM median values, younger age, and MASLD based on HVPG, MASLD, male sex, and HVPG were associated factors. The Kaplan-Meier survival estimates showed a trend towards reduced life expectancy for patients with HVPG≥12 and SSM≥85 kPa.
**Conclusions:** SSM@100hz, mirroring portal hypertension, can predict the de novo HCC occurrence in patients with cACLD with a follow-up time of 5 years.

## Figure:

| Variables | Characteristic of patients | | Competing-risks regression analysis | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Univariate analysis | | Multivariate HVPG-based model | | Multivariate SSM100Hz-based model | |
| | No HCC (n=45; 65%) | HCC occurrence (n=24; 35%) | Hazard Ratio (95% CI) | p-value | Hazard Ratio. (95% CI) | p-value | Hazard Ratio (95% CI) | p-value |
| Age | 62.9 (50.4-70.3) | 56.4 (49.1-68.3) | 0.979 (0.942 - 1.018) | 0.292 | 0.947 (0.906 - 0.991) | **0.018** | | |
| BMI (kg/m2) | 25.0 (24.0-26.4) | 30.0 (29.0-31.8) | 1.722 (1.331 - 2.227) | **<0.001** | | | | |
| Sex | | | 2.644 (0.861 - 8.121) | 0.087 | | | 10.296 (1.824 - 58.1) | 0.008 |
| *Female* | 14 (31.1%) | 3 (12.5%) | | | | | | |
| *Male* | 31 (68.9%) | 21 (87.5%) | | | | | | |
| **Aetiology** | | | | | | | | |
| MASLD | | | 5.234 ( 2.255 - 12.149) | **<0.001** | 20.911 (5.639 - 77.547) | **<0.001** | 18.351 (4.592 - 72.9669 | <0.001 |
| *Non metabolic etiology* | 38 (84.4%) | 8 (33.3%) | | | | | | |
| *Metabolic etiology* | 7 (15.6%) | 16 (66.7%) | | | | | | |
| Viral etiology | | | 1.134 (0.399 - 3.22) | 0.812 | | | | |
| *Non viral etiology* | 7 (15.6%) | 4 (16.7%) | | | | | | |
| *Viral etiology* | 38 (84.4%) | 20 (83.3%) | | | | | | |
| ALD | | | 0.609 (0.228 - 1.6323) | 0.325 | | | 0.223 (0.061 - 0.811) | 0.023 |
| *Non alcoholic etiology* | 35 (77.8%) | 20 (83.3%) | | | | | | |
| *Alcoholic etiology* | 10 (22.2%) | 4 (16.7%) | | | | | | |
| Esophageal Varices | | | 1.118 (0.596 - 2.098) | 0.727 | | | | |
| *Absent* | 17 (37.8%) | 9 (37.5%) | | | | | | |
| *F1-F2* | 22 (48.9%) | 14 (58.3%) | | | | | | |
| *F3* | 6 (13.3%) | 1 (4.2%) | | | | | | |
| Bilirubin | 0.9 (0.6-1.6) | 0.9 (0.8-1.5) | 0.900 (0.737 - 1.268) | 0.809 | | | | |
| Albumin | 4.0 (3.5-4.1) | 3.8 (3.5-4.2) | 0.967 (0.686 - 2.4623) | 0.422 | | | | |
| INR | 1.2 (1.1-1.3) | 1.2 (1.1-1.3) | 0.715 (0.1607 - 3.1897) | 0.661 | | | | |
| PLT | 88.0 (70.0-143.0) | 100.0 (61.0-126.5) | 0.994 (.985 - 1.003) | 0.198 | | | | |
| LSM median (kPa) | 23.4 (16.8-34.1) | 26.6 (17.6-35.3) | 1.02 (0.989 - 1.051) | 0.799 | | | | |
| SSM@100Hz median (kPa) | 53.6 (38.8-66.7) | 56.4 (40.4-78.9) | 1.022 (1.003 - 1.042) | **0.025** | 1.04 (1.013 - 1.066) | **0.003** | | |
| HVPG (mmHg) | 11.0 (9.0-14.0) | 13.5 (10.0-17.5) | 1.137 (1.047 - 1.235) | **0.002** | | | 1.171 (1.021 - 1.344) | **0.024** |
| Baveno VII Model - Rule IN | | | 2.165 (0.936 - 5.009) | 0.071 | | | | |
| *Absent* | 24 (53.3%) | 9 (37.5%) | | | | | | |
| *Present* | 21 (46.7%) | 15 (62.5%) | | | | | | |
| Baveno VII-SSM model (single cutoff) by SSM@100Hz - Rule IN | | | 4.329 ( 0.985 - 19.026) | **0.05** | | | | |
| *Absent* | 13 (28.9%) | 2 (8.3%) | | | | | | |
| *Present* | 32 (71.1%) | 22 (91.7%) | | | | | | |
| Baveno VII-SSM model (dual cutoff) by SSM@100Hz - Rule IN | | | 1.967 (0.834 - 4.635) | 0.122 | | | | |
| *Absent* | 17 (37.8%) | 7 (29.2%) | | | | | | |
| *Present* | 28 (62.2%) | 17 (70.8%) | | | | | | |

**Note:**

**Competing-risks events:** Liver Transplantation and/or Death (n=3)

**Baveno VII-SSM model (single cutoff) by SSM@100Hz**: two out of LSM ≥25 kPa; Platelet count <150 × 109 per L; SSM >40 kPa

**Baveno VII-SSM model (dual cutoff) by SSM@100Hz**: two out of:LSM ≥25 kPa; Platelet count <150 × 109 per L; SSM >50 kPa

**Baveno VII Model:** LSM ≥25 kPa

| | |
|---|---|
| **AUROC = 0.753** | **AUROC = 0.721** |
| **AIC = 94.423** | **AIC = 94.877** |
| **BIC = 100.66** | **BIC = 103.19** |

# PO6-12-YI
# Dissociation between acute liver decompensation and radiological response in patients with hepatocellular carcinoma treated with atezolizumab-bevacizumab

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**Background and aims:** The combination of atezolizumab (anti-PD-L1) and bevacizumab (antiangiogenic) (Atezo-Bev) is the first line treatment for advanced hepatocellular carcinoma (aHCC). This study analysed the incidence of acute liver decompensation (ALD) in patients treated with Atezo-Bev and its correlation with oncologic response.

**Method:** We included patients treated with Atezo-Bev in our centre from December 2020 to June 2023 with an available CT scan at 12 weeks (T1) from treatment start. Radiological response (RR) was defined according to mRECIST criteria. We considered *'responders'* patients with stable disease (SD) or partial response (PR) at T1. ALD was defined as the occurrence of ascites (grade ≥2), hepatic encephalopathy (grade ≥2), or variceal bleeding during treatment.

**Results:** Thirty-two patients were included, 78.1% were male with a mean age of 70.5 years. 25 patients had cirrhosis (78.1%), with etiologies including HCV (43.8%), HBV (12.5%), alcohol use disorder (40.6%), and MASLD (31.3%). Eight patients (25% of the total, 35% of cirrhotic) developed ALD during Atezo-Bev treatment. All 8 patients had cirrhosis and seven of them (87.5%) were in Child-Pugh class A. Within the general population, the response rate after 12 weeks was 62.5% (PR in nine cases, SD in the remaining 11). On the other hand, the entirety of the decompensated patients were responders at T1: three presented PR and five SD. Comparing patients with and without decompensation (8 vs 24), the rate of response resulted significantly different (100% vs 50%; p = 0.014). The median variation of alpha-fetoprotein levels at T1 was significantly different comparing responders and non-responders (p = 0.032).

**Conclusion:** Occurrence of ALD is frequent in cirrhotic patients during Atezo-Bev treatment and it is known to be associated with poor prognosis. Whilst the RR in the general population was 62, 5%, the entirety of patients who developed a decompensation were responders at T1. Despite the small size of our sample, the data suggest a pathogenetic link between tumour lysis and hepatic function deterioration possibly mediated by the inflammatory response.

# PO6-13-YI
# The prognostic impact of viral and non-viral etiologies on advanced hepatocellular carcinoma patients treated with atezolizumab plus bevacizumab: a real-world, multicenter study

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**Background and aims:** The impact of etiology on response to immunotherapy in advanced hepatocellular carcinoma (HCC) is being extensively debated. Early clinical trials suggested that PD-1/PD-L1 blockade prolonged survival of viral HCC patients, but not of those with non-viral HCC. However, a recent post-hoc analysis of the IMbrave-150 trial showed atezolizumab plus bevacizumab (A+B) to exhibit similar efficacy in viral and non-viral groups. Here we investigated the role of etiology in A+B-treated HCC patients in a real-world scenario to consolidate these findings and integrate them with previous comparative studies with Lenvatinib

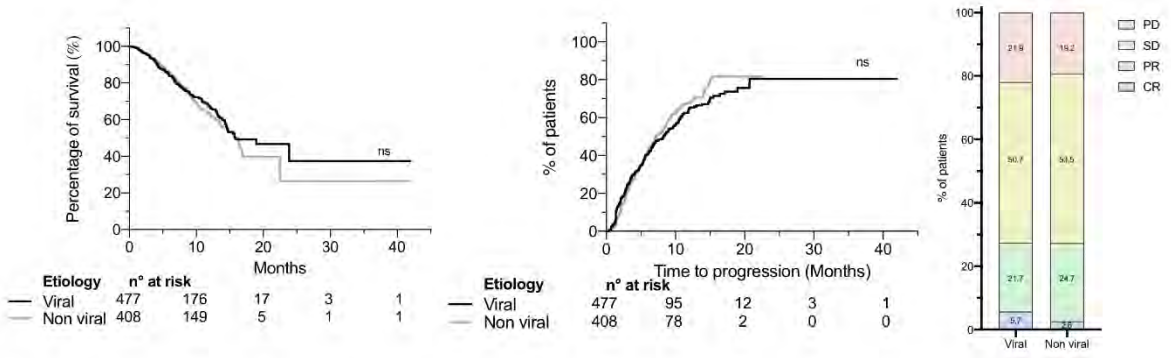
.

**Method:** We retrospectively analyzed 885 HCC patients treated with first-line A+B from Eastern and Western countries, 53.9% having viral and 46.1% non-viral etiology. Baseline clinical and laboratory characteristics were analyzed with uni- and multi-variate models to assess the impact of etiology on overall survival (OS), time to progression (TTP), response rates and to identify prognostic factors in etiology subgroups.

**Results:** Overall, no statistically significant differences were found in OS (mOS: viral 15.9months; non-viral 16.3months; p = 0.5464), TTP (mTTP: viral 8.3months; non-viral 7.2months; p = 0.4030), and response rates based on etiology (p = 0.0985). However, certain prognostic factors differed between the two groups. Among patients with viral etiology, eosinophil count ≥70/mL positively correlated with OS (p = 0.0310), while among non-viral patients aspartate transaminase (AST)<65 IU/L and alkaline phosphatase (ALP)≤125 IU/L levels were significantly related to OS (p = 0.0264 and p = 0.0432, respectively). Alpha-fetoprotein (AFP), neutrophil-to-lymphocyte ratio (NLR), and ALBI score were prognostic for both groups. The toxicity profile of A+B almost overlaps in the two etiology subgroups. Finally, we investigated the access to second-line treatments at progression of viral and non-viral patients, finding no significant difference based on disease etiology (p = 0.7153)

**Conclusion:** These findings indicate that underlying etiology does not significantly impact the outcome of HCC patients treated with A+B. However, some prognostic factors differ between viral and non-viral patients, with immunological factors being mostly related to the former while metabolic to the latter, supporting potential biological differences. This may also account for different outcomes of A+B observed in comparative studies with lenvatinib in etiology subgroups. Prospective and comparative trials stratifying by etiology are warranted to validate these findings and guide clinical decisions.

**Figure:**


Percentage of survival (%)
100
80
60
40
20
0
0
10
20
30
40
Months
ns
Etiology
n° at risk
Viral 477 176 17 3 1
Non viral 408 149 5 1 1
% of patients
100
80
60
40
20
0
0
10
20
30
40
Time to progression (Months)
ns
Etiology
n° at risk
Viral 477 95 12 3 1
Non viral 408 78 2 0 0
% of patients
100
80
60
40
20
0
21.9
15.2
50.7
53.5
21.7
24.7
5.7
3.8
Viral
Non viral
PD
SD
PR
CR

# PO6-16-YI
# Safety profile of the add-on combination of Regorafenib with Nivolumab after treatment with atezolizumab-bevacizumab in treated patients with hepatocellular carcinoma

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**Background and aims:** The combination of atezolizumab with bevacizumab (AB) is approved in Spain for patients with naïve hepatocellular carcinoma (HCC) under systemic treatment. However, second-line treatment of this population is an unmet need. The GOING (NCT04170556) trial is an investigator-initiated phase I/IIa study evaluating the safety of the sequential combination of Regorafenib (Rego) and Nivolumab (Nivo) in the second line after AB and sorafenib. Our objective is to report the safety profile of the Rego-Nivo combination in patients treated with AB (cohort B of the GOING clinical trial).

**Method:** Patients treated at least 2 cycles of AB or sorafenib and who have definitively discontinued these treatments. Patients received Rego monotherapy for the first 2 cycles (initial dose 160 mg/day, 3 weeks of treatment with 1 week off, dose adjusted for adverse events [AEs]) and Nivo is introduced on day 1 of cycle 3 (240 mg every two weeks). Treatment continues until unacceptable AEs, symptomatic progression, patient decision, or death.

**Results:** A total of 69 patients were included, 53 after sorafenib and 16 patients after AB, one patient did not receive any dose of Rego-Nivo. The safety of patients previously treated with sorafenib has been previously reported (AEEH 2021). Specifically, 53.4% of the 15 patients treated with AB were cirrhotic and 73% BCLC-C. The median overall follow-up in cohort B during the trial was 9.2 (range 3.5-15.8) months.

All patients in cohort B developed at least one AE, 6 (40%) had a serious AE, none related to Rego/Nivo, and 1 of them was grade 5. Ten (66.6%) patients of Cohort B died, 9 due to disease progression (90%) and 1 due to severe AE unrelated to the study treatments (pneumonia).

**Conclusion:** This initial data suggests that Rego-Nivo can be safely administered. Final analysis including efficacy results will be presented later.

# PO6-18-YI
# High prevalence of HCC among a diverse cohort of recently deceased persons with HCV

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**Background and aims:** It is estimated that for patients with hepatitis C virus (HCV) cirrhosis, the risk of hepatocellular carcinoma (HCC) is 1-4% per year and 5% per lifetime but achieving sustained virological response (SVR) with direct acting antivirals (DAAs) reduces the risk by approximately 70%. However, treatment rates have not reached target levels and are actually declining. Real-world evidence on the prevalence of HCC in current HCV patients in the DAA era is needed.

**Method:** We searched for all patients with current/prior HCV in a large urban tertiary care health system. Using the electronic medical record, we included all unique alive patients seen in the prior 3-years, with at least 1 of: positive HCV antibody, detectable HCV RNA, prior HCV medication, or ICD 9/10 code for HCV. We ran the query in 2022 and again in 2023. Next, we identified patients in the 2022 cohort who died before the 2023 cohort. We then extracted liver-related outcomes in the deceased by ICD 9/10 code: HCC, cirrhosis, varices, ascites, hepatorenal syndrome, and hepatic encephalopathy. For patients with HCC codes, we manually chart reviewed each to confirm HCC. We calculated descriptive statistics and compared characteristics of patients with and without HCC (Table 1). Categorical and continuous variables were analyzed using chi-square analysis and student t-test, respectively.

**Results:** Our search identified n = 6, 334 with current/prior HCV in 2022 and n = 4, 344 in 2023. We found n = 149 patients died between 2022 and 2023, the deceased HCV cohort. The average age at death was 66.4 years (SD 10.9), and patients were 61% male, 46% White, 23% Latinx and 14% Black. We found n = 43 had ICD code for HCC; after manual chart review, n = 42 had confirmed HCC. We also found codes for cirrhosis (28%), ascites (24%), and gastroesophageal varices (21%) in this population (Table 1). The deceased HCV patients had an extremely low rate of HCV treatment (28%) and SVR (18%) (Table 1).

**Conclusion:** This real-world study found 28% of recently deceased HCV patients had associated HCC, an extremely high prevalence, on average much younger than U.S. lifespan. These HCC cases and deaths are occurring almost one decade after all oral DAAs became available, yet only 28% and 18% of the cohort had been treated and had achieved SVR, respectively. Although the study size is too small to determine independent risk factors for HCC, this high prevalence of HCC and low prevalence of treatment, supports the urgent need for efforts to increase treatment of HCV patients to prevent HCC.

**Figure:**

Baseline characteristics of the overall cohort of deceased patients and those with and without HCC

| | Total (n = 149) | No HCC (n = 107) | HCC (n = 42) | P-value |
|---|---|---|---|---|
| Age (mean ± stddev) | 66.4 ± 10.9 | 65 ± 12 | 70 ± 6.2 | **0.0108** |
| Gender | | | | 0.104 |
| Male | 91 (61%) | 61 (57%) | 30 (71%) | |
| Female | 58 (39%) | 46 (43%) | 12 (29%) | |
| Race | | | | 0.448 |
| White | 69 (46%) | 46 (43%) | 23 (55%) | |
| Black | 22 (14%) | 18 (17%) | 4 (9%) | |
| Latinx | 34 (23%) | 24 (22%) | 10 (24%) | |
| Other | 24 (16%) | 19 (18%) | 5 (12%) | |
| Language | | | | 0.178 |
| English | 134 (90%) | 94 (88%) | 40 (95%) | |
| Non-English | 15 (10%) | 13 (12%) | 2 (5%) | |
| PCP | 18 (12%) | 16 (15%) | 2 (5%) | 0.086 |
| Ever hepatology visit | 77 (52%) | 41 (38%) | 36 (86%) | **< 0.0001** |
| Ever HCV diagnosis by ICD 9/10 | 100 (67%) | 60 (56%) | 40 (95%) | **< 0.0001** |
| Prior treatment | 41 (28%) | 23 (21%) | 18 (43%) | **0.009** |
| SVR | 27 (18%) | 14 (13%) | 13 (31%) | **0.011** |
| Ascites | 36 (24%) | 1 (0.9%) | 35 (83%) | **<0.0001** |
| Varices | 31 (21%) | 13 (12%) | 18 (43%) | **<0.0001** |
| Cirrhosis | 42 (28%) | 1 (0.9%) | 41 (98%) | **<0.0001** |

# PO7-01
# α-FAtE: a new predictive score of response to atezolizumab plus bevacizumab for unresectable hepatocellular carcinoma incorporating α-Fetoprotein, Alkaline phosphatase and Eosinophil count

Federico Rossari[1], Toshifumi Tada[2], Shimose Shigeo[3], Masatoshi Kudo[4], Changhoon Yoo[5], Jaekyung Cheon[6], Fabian Finkelmeier[7], Ho Yeong Lim[8], José Presa[9], Gianluca Masi[10], Francesca Bergamo[11], Elisabeth Amadeo[1], Francesco Vitiello[1], Mara Persano[12], Silvia Foti[1], Fabio Piscaglia[13], Mario Scartozzi[12], Stefano Cascinu[1], Margherita Rimini[1], Andrea Casadei Giardini[1]

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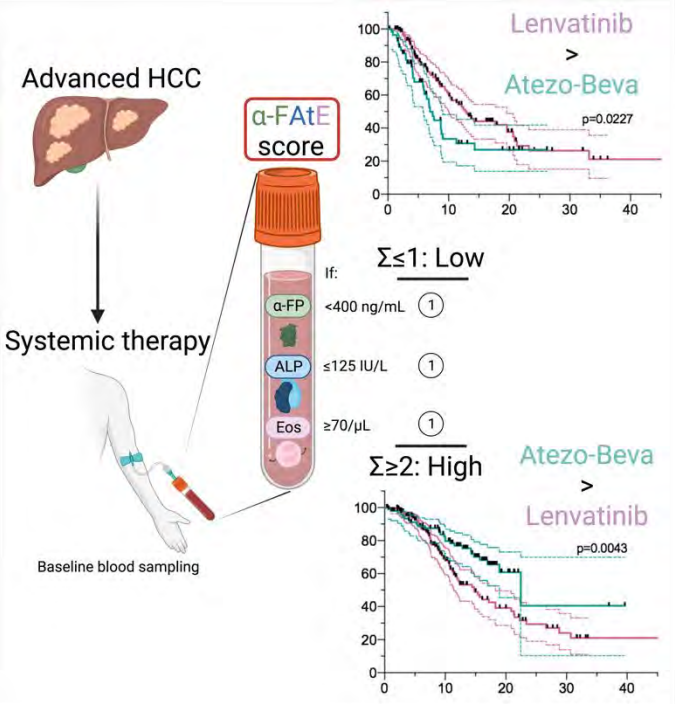
**Background and aims:** Atezolizumab plus bevacizumab (A+B) and lenvatinib can be alternatively used as first-line systemic treatment of unresectable hepatocellular carcinoma (HCC). However, no direct comparison of the two regimens has been performed in randomized clinical trials, making the identification of baseline differential predictors of response of major relevance to tailor the best therapeutic option to each patient.

**Method:** Baseline clinical and laboratory characteristics of real-world first-line A+B-treated HCC patients (n = 204) were analyzed in uni- and multi-variate analyses to find potential prognostic factors of overall survival (OS). Significant variables were incorporated in a composite score (α–FAtE) and it was tested for specificity and sensitivity in receiver operating characteristic (ROC) curve and then in multivariate analysis for OS. The score was applied in uni- and multi-variate analyses for OS of a comparable first-line lenvatinib-treated HCC population (n = 339). Finally, comparison between treatments was performed in patients with low and high □-FAtE scores and predictivity estimated by interaction analysis. Time-to-progression (TTP) was a secondary end point.

**Results:** OS of A+B-treated HCC patients was significantly longer in those with α–fetoprotein <400ng/ml (HR 0.62, p = 0.0407), alkaline phosphatase (ALP) <125U/L (HR 0.52, p = 0.0189) and eosinophil count ≥70/mL (HR 0.46, p = 0.0013). The α–FAtE score was generated by the sum of single points attributed to each variable among the above reported. In ROC curve analysis, superior sensitivity and specificity were achieved by the score compared to individual variables (AUC 0.794, p <0.02). High-score patients had longer OS (HR 0.44, p = 0.0009) and TTP (HR 0.34, p <0.0001) compared to low-score onnes if treated with A+B, but not with lenvatinib. Overall, A+B was superior to lenvatinb in high-score patients (HR 0.55, p = 0.0043) and inferior in low-score ones (HR 1.75, p = 0.0227). At interaction test, low α–FAtE score resulted as negative predictive factor of response to A+B (p = 0.0004).

**Conclusion:** α–FAtE is a novel prognostic and predictive score of response to first-line A+B for HCC patients that, if validated in prospective studies, could drive therapeutic choice between lenvatinib and A+B as first-line systemic treatment.

**Figure:**


Advanced HCC
Systemic therapy
Baseline blood sampling
α-FAtE score
α-FP
ALP
Eos
If:
<400 ng/mL 1
≤125 IU/L 1
≥70/μL 1
Σ≤1: Low
Σ≥2: High
Lenvatinib > Atezo-Beva
p=0.0227
Atezo-Beva > Lenvatinib
p=0.0043

# PO7-03-YI
# Better end points of outcome comparisons between HAIC and Sorafenib in advanced hepatocellular carcinoma: a systematic review and meta-analysis

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**Background and aims:** Overall survival (OS) is the generally accepted end point for clinical trials. However, it is difficult to get sufficient follow-up from patients with advanced hepatocellular carcinoma (HCC). Recently, intermediate end points such as objective response rate (ORR), disease control rate (DCR), and progression-free survival (PFS) are used in clinical trials to evaluate treatment efficacy. This study aims to find surrogate end points of OS to assess the clinical benefits between hepatic artery infusion chemotherapy (HAIC) and Sorafenib in treating HCC.

**Method:** A rigorous systematic literature search was conducted to collect available studies comparing patients' outcome differences between HAIC and sorafenib. The protocol was available at PROSPERO (CRD42023458845).

**Results:** A total of 26 studies with 6456 patients (HAIC:2648; Sorafenib:3808) were included. The correlation analysis showed that PFS had a strong association with OS ($R^2$ = 0.8073, 95%CI [0.67, 0.97]), 1y-PFS showed good fitness with general PFS ($R^2$ = 0.9746, 95%CI [0.96, 0.99]). In addition, it is closely associated with both general OS ($R^2$ = 0.7708, 95%CI [0.61, 0.97]) and 1y-OS ($R^2$ = 0.7208, 95%CI [0.56, 0.95]). No statistical association between DCR/ORR with general OS or PFS was found though 1y-OS ($R^2$ = 0.3080, 95%CI [-0.84, -0.05]) and 1-y PFS ($R^2$ = 0.3163, 95%CI [-0.82, -0.08]) were statistically correlated with DCR. Pooling data after propensity score matching (PSM) also confirmed that 1y-PFS had correlations with final clinical outcomes (PFS: $R^2$ = 0.9847, 95%CI [0.89, 0.99]; 1y-OS: $R^2$ = 0.8804, 95%CI [0.24, 0.98]; OS: $R^2$ = 0.923, 95%CI [0.45, 0.99])

**Conclusions:**The study showed that both 1y-PFS and PSM had strong associations with clinical outcomes after HAIC or Sorafenib treatment in HCC. This study has provided a feasible tool for designing clinical trials directly comparing HAIC and Sorafenib treatment for HCC.

# PO7-04-YI
# The detrimental effect of metabolic risk factors on gender differences in HCC development and treatment allocation

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**Background and aims:** Due to the liver's susceptibility to sexual hormones, the incidence and severity of hepatocellular carcinoma (HCC) differ between males and females. Metabolic comorbidities, such as obesity and diabetes, can increase the risk of HCC development, acting as pro-oncogenic risk factors. Despite obesity being more frequent in women, men with obesity had a higher probability of developing HCC compared to women with obesity. The interaction between gender and metabolic risk factors on HCC development and progression is not fully understood. The aim of this study is to evaluate the differences in HCC features and staging in males and females according to the presence of associated metabolic comorbidities.

**Methods:** We retrospectively evaluated 126 patients followed from 2014 to 2020, assessing patient staging and treatment decisions using the Barcelona Clinic Liver Cancer (BCLC) system. A platelet count <150, 000mm³ suggested portal hypertension in 65% of cases. 14% underwent surgery, 74% received locoregional therapy, 3% systemic therapy, and 8% received best supportive care.

**Results:** HCC had a higher prevalence in men compared to women (78% vs. 22%, respectively), with a significantly lower mean onset age in men (67 ± 11 vs. 74 ± 11, p = 0.04). The main etiology was related to hepatitis C virus (HCV) infection in both genders (41% in men vs. 70% in women, p = 0.03). Men exhibited a greater coexistence of multiple known pathogenic factors promoting HCC, including diabetes (43% in men vs. 20% in women, p = 0.04) and obesity (64% in men vs. 38% in women, p = 0.09). Additionally, men had a greater number of lesions at onset (52% in men vs. 80% in women, p = 0.03), with a similar lesion size. In the presence of coexisting metabolic comorbidities, compared to patients without them, a higher percentage of patients with low platelets (71% vs 56%, p = 0.03) and higher bilirubin (36% vs 15%, p = 0.04) was observed, irrespective of gender. Among treatments, patients with metabolic risk factors tended more towards locoregional treatment rather than resection (52% vs. 26%), although statistical significance was not reached (p = 0.08).In multivariate analysis, the coexistence of metabolic risk factors strongly correlated with male gender (OR 10.8, p = 0.04), low platelet levels (OR 6.7, p = 0.03), and high bilirubin levels (OR 4.95, p = 0.04), independently of age. Nevertheless, the multifocality of HCC at onset appears to correlate with male gender (OR 10.5, p = 0.04).

**Conclusion:** Our study reveals a strong association between metabolic risk factors and male gender. The increased incidence of diabetes and obesity leads to a more severe liver disease, expressed by increased portal hypertension and bilirubin levels, and less curative treatments. Early detection and treatment of metabolic comorbidities are crucial to reduce the risk of disease progression and enable patients to undergo a more curative HCC treatment.

# PO7-06-YI
# Pre-treatment serum N-glycans predict poor immunotherapy response and survival in hepatocellular carcinoma

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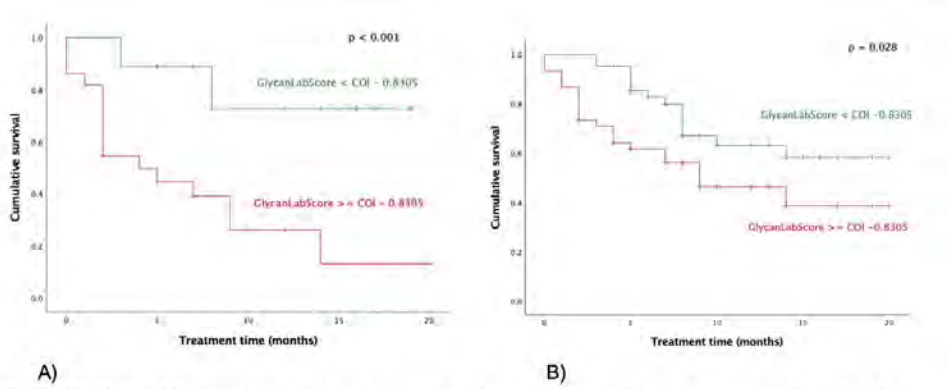
**Background and aims:** Patients with hepatocellular carcinoma (HCC) frequently present with advanced disease, for which immunotherapy is the most promising first-line agent-despite the fact that only a quarter of patients show a favourable response. Although serum N-glycans (glycomics) are altered in HCC, they have not been assessed as a predictive marker for treatment response. We investigated glycomics as a predictive indicator of poor response to immunotherapy and associated survival in advanced HCC.

**Method:** A total of 90 immunotherapy-naive HCC patients were retrospectively recruited with a pre-treatment serum sample. Whole serum N-glycomic analysis was performed using the optimised 96-well on-membrane deglycosylation technique (DSA-FACE). The univariate analysis of survival was calculated by the Kaplan-Meier method and compared by log-rank test. A multivariate Cox proportional hazards model was built to identify independent predictive factors.

**Results:** This cohort included 90 HCC patients receiving atezolizumab and bevacizumab combination therapy, of which 40 of them had no prior locoregional treatment and were thus completely treatment-naive. Pre-treatment NA3Fc and NA3Fbc glycans were significantly increased in patients who showed poor response (progressive disease) after a median treatment time of 5.9 months as compared with patients who did not. Other independent risk factors were decreased hemoglobin (Hb) and increased aspartate aminotransferase (AST) pre-treatment levels. For the complete treatment-naive patients (n = 40) with a baseline GlycanScore (compositescore of NA3Fc and NA3Fbc) above the cut-off value of 1.75, the HR for poor response was 2.16 (95% CI [1.36-3.42], p = 0.001). Combination of this GlycanScore with Hb and AST resulted in the GlycanLabScore with an HR of 2.72 (95% CI [1.677-4.401], p <0.001) for patients at risk of poor response with low survival (p <0.001), exceeding the cut-off value of -0.83. Similar findings could be retained when the total cohort was considered, thus including the 50 patients who did receive prior locoregional treatment before the start of immunotherapy. In these patients, the risk for having progressive disease with low survival (p = 0.0017, p = 0.028) was related to a GlycanScore with HR of 1.82 (95% CI [1.40-2.37], p <0.001) and a GlycanLabScore with HR of 2.63 (95% CI [1.91-3.63], p <0.001), based on the same cut-off values. In comparison, the alphafetoprotein (AFP) level was not able to significantly differentiate between treatment response, nor could it be included as an independent predictor in the cox regression model for survival prediction.

**Conclusion:** In this study, we demonstrated that pre-treatment tri-antennary core and branch fucosylated glycans, combined or not with Hb and AST levels, are predictive of poor immunotherapy response and low survival in advanced HCC.

**Figure:**




A) B)

**Figure 1.** Kaplan-Meier survival curve in patients with poor immuno-therapy response according to the GlycanLabScore in the complete treatment-naive cohort, n=40 (A) and immunotherapy-naive cohort, n=90 (B).

# PO7-07
# Prophylaxis of variceal bleeding in patients receiving atezolizumab-bevacizumab for hepatocellular carcinoma

Francesco Tovoli[1], Rusi Chen[1], Caterina Vivaldi[2], Piera Federico[3], Andrea Palloni[4], Andrea Dalbeni[5], Caterina Soldà[6], Ingrid Garajovà[7], Benedetta Stefanini[1], Luca Ielasi[8], Stefania De Lorenzo[9], Alessandro Granito[1], Maria Boe[1], Gianluca Masi[2], Sara Lonardi[6], Giovanni Brandi[1], Bruno Daniele[3], David Sacerdoti[5], Lorenzo Lani[1], Gianluca Svegliati-Baroni[10], Claudia Campani[11], Fabio Piscaglia[1]

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**Background and aims:** Guidelines recommend atezolizumab/bevacizumab (AB) as a frontline therapy for patients with unresectable hepatocellular carcinoma (HCC). Bevacizumab may increase the risk of bleeding. Patients with cirrhosis should undergo an upper digestive endoscopy (EGDS) prior to the start of AB. Moreover, patients with neoplastic portal vein invasion (nPVT) may develop portal hypertension even in the absence of cirrhosis.We aimed to report the prevalence of esophageal varices in patients undergoing AB for unresectable HCC, identify risk factors associated to the presence of varices, describe prophylaxis measures and the prevalence of bleeding.

**Method:** The ARTE database include prospectively-collected from patients treated with AB in a real-life setting. We explored clinical data and outcome of HCC patients included in this database (March 2022-November 2023).

**Results:** Data of 157 patients from 12 centres were collected (median follow-up 8.9 months). Most patients (n = 114, 72.4%) had liver cirrhosis. Overall, 117 patients (74.5%) had received an EGDS <6 months before starting AB. Amongst them, 34 (29.1%) had esophageal varices. Prophylaxis of bleeding was performed as followed: non-selective beta-blockers (NSBB) [n = 17, 50.0%], elastic band ligation (EBL) [n = 2, 5.9%], NSBB+EBL (n = 3, 8.8%). Twelve patients (35.3%) did not receive prophylaxis for absolute or relative contraindications. There was no significant difference in the management between hepatology and oncology centres (p = 0.662). The presence of varices was independently predicted by platelet count<150.000/mmc (OR 4.7, 95% CI 1.8-12.2, p = 0.001) and alcoholic etiology (OR 4.2, 95% CI 1.6-11.0, p = 0.004). Neither ALBI grade>1 (OR 1.6, 95% CI 0.6-4.22) or nPVT of the main portal trunk (OR 2.0, 95% C 0.74-9.6) reached the full statistical significance. Variceal bleeding occurred in 4 patients (2.6%; G3: n = 1; G4:n = 2; G5:n = 1).

**Conclusion:** Variceal bleeding under AB remains a rare occurrence, but with severe consequences. EGDS should be strongly recommended for cirrhotic patients, especially those with low platelet count and/or alcoholic aetiology.

# PO7-08-YI
# Do ACE inhibitors have a role in preventing drug-related proteinuria in advanced HCC patients?

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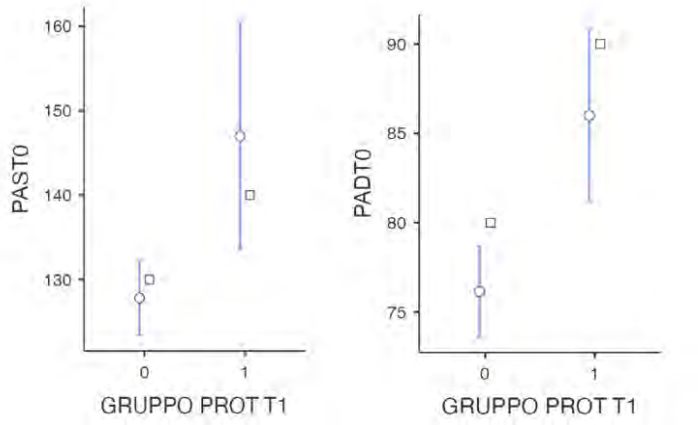
**Background and aims:** Both tyrosine kinase inhibitors (TKI) and Atezolizumab plus Bevacizumab (A+B) are used as systemic anti-cancer treatment for hepatocellular carcinoma (HCC). Hypertension and proteinuria are among most common side effects caused by both drugs and can lead to treatment discontinuation. Since Angiotensin-Converting Enzyme (ACE) inhibitors are known to reduce proteinuria, we aimed to verify if they have a protective role on the development of proteinuria and whether any other factor was associated to these adverse events.

**Method:** We retrospectively included consecutive patients receiving systemic therapy as standard of care for non-resectable HCC from 3 different Italian centers. We verified the prevalence of proteinuria within 3 months from the start of treatment with either TKI or A+BDifferences between basal characteristics of the group of patients who developed proteinuria versus patients who did not were analyzed using a Mann-Whitney test or $X^2$.A regression analysis was performed to find potential predictors of proteinuria.

**Results:** A total of 151 patients were analyzed (54 receiving A+B, 97 receiving TKI). Significant proteinuria developed in 29 (19.2%) of the patients without significant differences between different treatments. No difference was observed in terms of history of hypertension or anti-hypertensive drug across the two groups. Only serum creatinine, systolic and diastolic blood pressure at the beginning of the treatment were significantly different ($p = 0.023$, $p = 0.014$, respectively).
Among patients who developed proteinuria, only a basal creatinine serum level and elevated systolic blood pressure (OR 1.14, $p = 0.048$) were independently associated to the development of the outcome.

**Conclusion:** Previous use of ACE inhibitors does not prevent proteinuria, however a scarce control of blood pressure at the beginning of systemic treatment is independently associated with its occurrence. An aggressive initial approach to lower systemic blood pressure seems justifiable.

**Figure:**


PAST0
160
150
140
130
0
1
GRUPPO PROT T1
PADT0
90
85
80
75
0
1
GRUPPO PROT T1

# PO7-09-YI
# Does AtezoBev present as a safe and efficacious treatment option for hepatocellular carcinoma in patients with Child-Pugh B cirrhosis? A retrospective multicentric real-world study

Leonardo Stella[1 2], Maria Pallozzi[2], Lucia Cerrito[1], Maurizio Pompili[1 2], Fabio Piscaglia[3 4], Francesco Tovoli[3 4], Fabio Marra[5 6], Campani Claudia[6], Elisa Pellegrini[6], Clemence Hollande[7], Sabrina Sidali[7], Antonio Gasbarrini[1 2], Mohamed Bouattour[7], Francesca Romana Ponziani[1 2]

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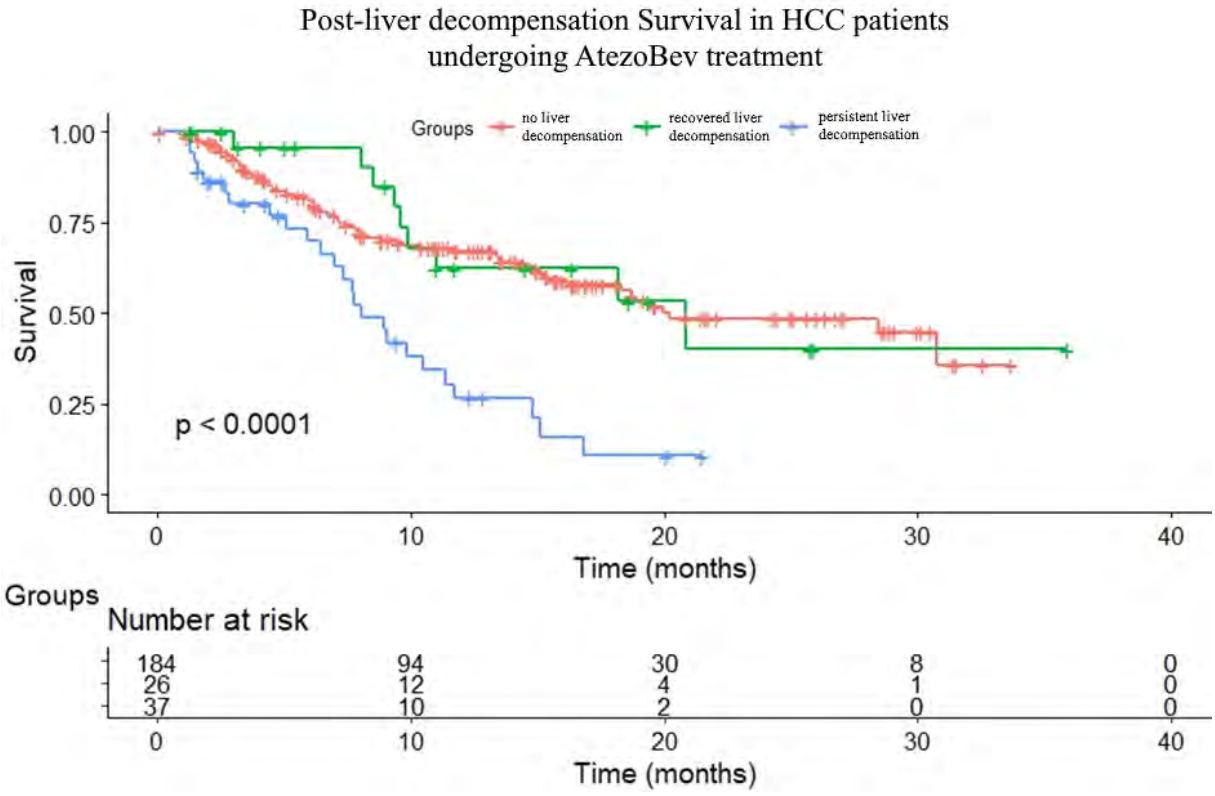
**Background and aims:** Initial management of unresectable hepatocellular carcinoma (HCC) involves the use of atezolizumab plus bevacizumab (AtezoBev). There is a lack of research examining the influence of hepatic decompensation on patients during treatment and its impact on survival.

**Method:**
Between 2018 and 2023, 247 patients diagnosed with unresectable HCC and eligible for AtezoBev treatment were enrolled. Liver function was graded for all patients, comparing Child Pugh A (CPA, 59.5%) to Child Pugh B (CPB, 17.4%) and non-cirrhotic (NC, 23.1%). A survival analysis assessed median overall survival (mOS), progression-free survival (PFS), and time-to-progression (TTP), while radiological response was evaluated using RECIST v1.1. Treatment-related adverse events (trAEs) graded according to CTCAE v5.0 were collected to evaluate AtezoBev safety.We defined time-to-decompensation (TTD) as the interval from treatment start to the occurrence of events associated with the loss of liver function or worsening of portal hypertension. Its role in assessing the safety of treatment in cirrhotic patients was evaluated. Then, mOS was assessed in patients who experienced decompensation compared to those who didn't modify liver function.

**Results:** AtezoBev demonstrated significantly better mOS (20.2 vs. 9.8 months, p <0.0001) and PFS (12.9 vs. 8.3 months, p <0.017) in CPA patients compared to CPB patients. However, there were no differences in TTP (16.3 vs 12.3 months, p = 0.14), overall response rate (ORR, 24.4% vs 18.6%, p = 0.46), and disease control rate (DCR, 56.4% vs. 55.8%, p = 0.93) between the two groups.
The incidence of treatment-related adverse events (trAEs) remained consistent across subgroups, except for portal hypertension-related events, which were more frequent in the CPB group.
Indeed, CPB patients experienced a higher incidence of liver decompensation events (50% vs. 27.8%, p = 0.006), resulting in a TTD of 9.1 months. In contrast, CPA patients didn't reach a 50% occurrence rate of decompensation events during the follow-up period. Among patients who experienced liver decompensation (35% CPB, 65% CPA), those who regained previous liver function (31% CPB, 69% CPA) achieved a mOS comparable to those who didn't undergo liver decompensation (20.9 months vs 20.2 months, p = 0.77). However, persistent loss of liver function (38% CPB, 62% CPA) resulted in a poorer prognosis (mOS 8.1 months).

**Conclusion:** AtezoBev demonstrated efficacy and safety in both CPA and CPB subgroups. Liver decompensation had a higher incidence in CPB groups, but patients who recover from a liver decompensation related event showed a mOS comparable to those who didn't suffer from it. Considering this, access to AtezoBev in routine practice should be considered for CPB patients under close monitoring. Additionally, TTD could serve as a novel safety outcome for cirrhotic patients undergoing systemic treatment.

**Figure:**


Post-liver decompensation Survival in HCC patients undergoing AtezoBev treatment
Groups
no liver decompensation
recovered liver decompensation
persistent liver decompensation
Survival
1.00
0.75
0.50
0.25
0.00
p < 0.0001
0
10
20
30
40
Time (months)
Groups
Number at risk
184 94 30 8 0
26 12 4 1 0
37 10 2 0 0
0
10
20
30
40
Time (months)

# PO7-10
# The impact of genomic alterations on response rate and survival outcomes in advanced BTC (biliary tract cancers) patients who receive cisplatin/gemcitabine plus durvalumab

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**BACKGROUND:** The TOPAZ-1 phase III trial reported a survival benefit with the anti-programmed death cell ligand 1 (anti-PD-L1) durvalumab in combination with cisplatin/gemcitabine in patients with advanced biliary tract cancer (BTC), but no data about the prognostic impact of genetic alterations in real-world setting are already available.

**METHODS:** A real-world population of advanced BTC patients who were studied by a 324-gene next generation sequencing (NGS) panel was considered for the present analysis. Patients were treated with cisplatin/gemcitabine plus durvalumab as first-line treatment at 11 Italian centers. The aim of the present analysis was to investigate the potential impact in terms of progression free survival (PFS) and overall survival (OS) of the most frequent genomic alterations. Moreover, a comparative genomic analysis between responding patients and non-responding patients was performed.

**RESULTS:** From February 2022 to November 2022, 51 patients were enrolled and studied with a 324-gene NGS panel. After a median follow-up of 6.2 months (95% CI 4.3-10.9), the median OS was not reached, whereas the median PFS was 8.5 months (95% CI 6.3-8.9). Most frequently altered genes were: ARID1A (29.5%), CDKN2A/CDKN2B (21.5%), MLL2 (17.5%), BAP1 (15.5%), BRCA2 (15.5%), PBRM1 (15.5%), TP53 (14%), IDH1 (12%), KRAS (12%) and MTAP (12%). Several other genes were reported to be altered in 10% of the patients, including ATM, MDM2, MSH3, PIK3CB and SMAD4. Of interest, 37% of the patients showed alterations in genes involved with the BRCAness phenotype. No genetic alterations were reported to have an impact on PFS or OS in our population. Overall, 47 patients were included in the response rate analysis. The investigator-assessed confirmed objective response rate (ORR) was 34%, and the disease control rate (DCR) was 80.9%. From the genetic point of view, a statistically significant difference in terms of prevalence of PBRM1 mutation was highlighted between responders and non-responders, since a higher prevalence of PBRM1 mutated patients was observed in the responders' group compared to non-responders' group (31% Vs 6.5%).

**CONCLUSION:** The present work reported the first genomic analysis on a cohort of patients who received cisplatin/gemcitabine plus durvalumab as first-line treatment in a real-world setting. Further investigation on larger number of patients is needed.

# PO7-11
# Early PIVKA-II decrease could possibly predict objective response in atezolizumab/bevacizumab (ATZ/BEV) treated cirrhotic patients with advanced proliferative hepatocellular carcinoma (HCC)

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**Background and aims:** Changes in serum biomarker levels such as alpha-fetoprotein (AFP) and protein-induced by vitamin-K absence (PIVKA-II) or scores such as Child- Pugh (CPT), MELD/Na and Crafity score (CS) during ATZ/BEV therapy have been recently correlated with treatment response in patients with HCC. Moreover, molecular classification of HCC is associated with specific clinical outcomes. We investigated the application of the above biomarkers and scores for assessment of response in white European cirrhotic patients with advanced HCC receiving ATZ/BEV therapy according to proliferative (P)/non proliferative (NP) histological status.

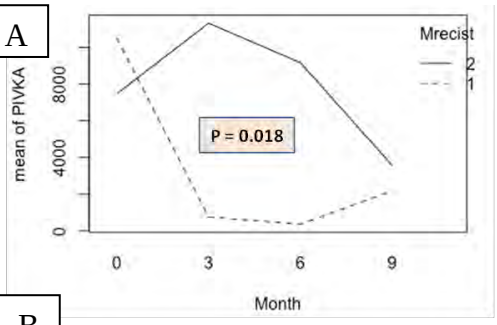
**Method:** 38 cirrhotic patients with histologically confirmed advanced HCC were included (32 males, mean age = 66, median MELD/Na = 8, 34 CPT-A, 19 ALBI-I, 13 CS-0). The mean duration of treatment was 9.5 months (10 treatment cycles). 21 of them were categorized as P and 17 as NP HCC, according to the currently proposed classification (Nault JC et al, J Hepatol 2018). 9 patients with complete or partial response according to mRECIST criteria were categorized as having an objective response (OR) and the rest of them as having a non-objective response (NOR). AFP, PIVKA-II, CPT, ALBI, CS and MELD/Na scores were evaluated in all patients at the first day of treatment and then every 3 months (3 cycles of therapy). 4 groups were then constructed: [A: P/OR (n = 7), B: P/NOR (n = 14), NP/OR (n = 2), NP/NOR (n = 15)], according to morpho molecular classification and response to therapy

**Results:** All 4 groups were comparable for all the baseline parameters evaluated. Mean values of biomarkers and scores were estimated for each group. We then compared these values during the same month between the 2 groups (OR, NOR) for each one of the two molecular classes (P, NP). Moreover, we compared these values during the same month between the two groups (OR, NOR) for the total study population. Interestingly, a statistically significant difference was observed only for biomarker PIVKA-II during the third month of therapy in the whole study population ($p = 0.018$) as well as in the P-HCC class ($p = 0.04$) between the OR vs NOR group. Similar findings were not observed in patients from the NP-HCC class ($p = 0.8$). AFP and clinical scores failed to show any advantage in the prediction of response. In the multivariate analysis, considering AFP, PIVKA-II, CPT, ALBI, CS and MELD/Na, a significant trend for predicting objective response was observed only for PIVKA-II levels at month 3 ($p = 0.093$).

**Conclusion:** PIVKA-II could be a useful biomarker for the prediction of OR in ATZ/BEV treated cirrhotic patients with advanced HCC, mainly in those with proliferative HCC subclass. These findings should be further evaluated in larger cohorts of patients.

**Figure:** Mean PIVKA-II values during ATZ/BEV treatment between patients with (dash line) or without (solid line) OR for the whole study population, the NP and the P-HCC class

A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8000
0
3
6
9
Month
Mrecist
2
1
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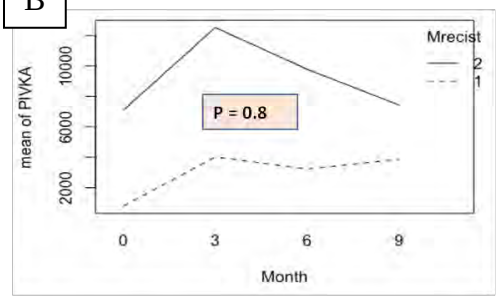
B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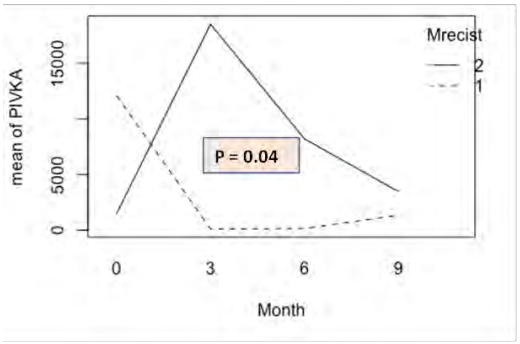
10000
0
3
6
9
Month
Mrecist
2
1
P = 0.8

C


mean of PIVKA
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5000
15000
0
3
6
9
Month
Mrecist
2
1
P = 0.04

# PO7-12
# Declining hepatitis C virus-related hepatocellular carcinoma mortality in the direct-acting antiviral therapy era in the United States, from 1999 to 2021

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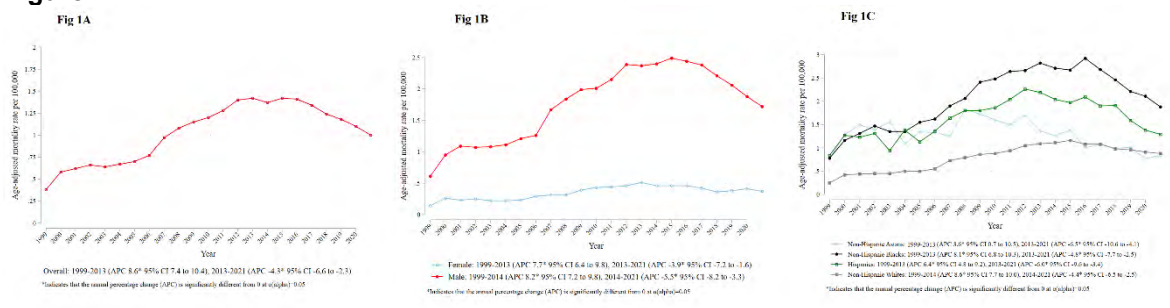
**Background and aims:** The risk for Hepatocellular Carcinoma (HCC) attributed to hepatitis C virus (HCV) infection has substantially decreased with the introduction of direct-acting antiviral (DAA) therapies in late 2013 leading to improvements in sustained virological response among patients. However, patients with advanced fibrosis and cirrhosis still remain at high risk for HCC incidence even after HCV clearance. Contemporary mortality trends in HCV-related HCC deaths in the DAA era have not been explored. Therefore, a population-based study using the national mortality database was performed.

**Method:** We utilized the Centers for Disease Control and Prevention Wide-Ranging, Online Data for Epidemiologic Research (CDC WONDER) database which provides information from death certificates of all United States residents according to the International Classification of Diseases, Tenth Revision (ICD-10). The mortality data were obtained for the adult population aged 35-85 years from January 1999 to December 2021. HCC (ICD-10 codes: C22.0) was listed as underlying or primary cause of death, and HCV (ICD-10 code: B18.2, B17.1) was listed as a contributing cause of death. Age-adjusted mortality rates (AAMR) per 100, 000 population were calculated by standardizing deaths to the 2000 US Standard population. Using Joinpoint regression program, we calculated the average annual percentage change (AAPC), along with 95% confidence intervals to analyze trends in mortality stratified by sex and race.

**Results:** 43, 822 deaths associated with HCV related HCC occurred between 1999 and 2021 among US adults. Overall, AAMRs for HCV-related HCC increased from 0.35 in 1999 to 1.47 in 2013 with an annual increase of 3.3% (95% CI, 3.1 to 3.5). However, a sharp decline was observed after the introduction of DAA from 1.37 in 2014 to 1.00 in 2021, with an annual decline of -4.3% (95% CI, -6.6 to -2.3). Males had consistently higher AAMR than females across all years. For males, AAMR increased by 8.2% annually from 1999-2014 but decreased by 5.5% annually from 2014-2021. In females, AAMR increased by 7.7% annually from 1992-2013 but decreased by 3.9% annually from 2013-2019. Compared with other race groups, non-Hispanic (NH) Blacks had a higher mortality rate (1.88 per 100, 000 versus 1.29 for Hispanics versus 0.88 for NH Whites and 0.84 for NH Asians in 2021). Over the entire study period, mortality rates decreased after 2013 for all races, the NH Asian population experienced the greatest decline in AAMR from 2013 to 2021 (AAPC: -6.5%; 95% CI, -10.6 to -4.1).

**Conclusion:** After the introduction of DAA-based treatments, HCV-related HCC mortality has decreased compared with the pre-DAA era. However, minorities in the United States have a disproportionately higher burden of HCV-related HCC mortality, highlighting the need to address disparities in access to healthcare and newer treatments in the United States.

**Figure:**



# PO7-13-YI
# The role of tumor molecular profiling in patients with advanced biliary tract cancer receiving systemic treatment

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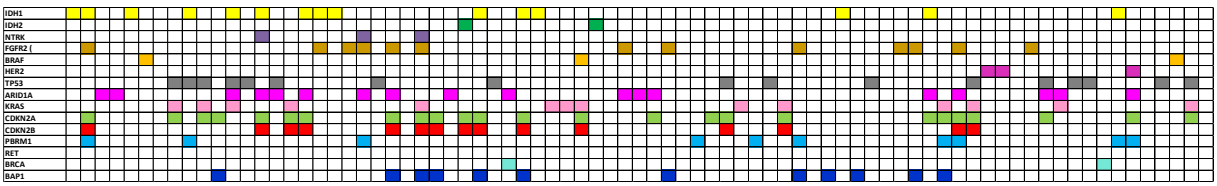
**Background and aims:** Next Generation Sequencing (NGS) has allowed the identification of several driver alterations in biliary tract cancer (BTC) with prognostic and predictive value. We aimed to provide an analysis of most common genetic alterations and assess their prognostic implications.

**Method:** We selected consecutive patients (pts) from a prospectively maintained dataset who were diagnosed with BTC between 2017 and 2023, with available NGS-based tumor molecular profiling, and who received at least one line of systemic treatment. Overall survival (OS) was calculated from date of diagnosis using the Kaplan-Meier method. Median OS (mOS) differences between pts with or without specific molecular alterations were evaluated using a log-rank test at a two-sided significance level of 0.05.

**Results:** 79 pts were included, mostly with intrahepatic cholangiocarcinoma (91%). 34 pts (43%) underwent surgery, and 71% of them received adjuvant therapy, 45 pts (57%) had de novo metastatic disease. 51 pts (65%) received cisplatin-based first-line systemic therapy. Most frequent alteration was CDKN2A mutation (25 pts, 32%), followed by ARID1A (18 pts, 23%), TP53 (17 pts, 22%), KRAS (15 pts, 19%), IDH1 (15 pts, 19%), and BAP1 mutations (12 pts, 15%). FGFR2 rearrangement was found in 7 pts (9%), while BRAF mutation and HER2 amplification were found in 3 (4%) and 2 (2%) pts. 36 pts (46%) had co-mutations (Figure). At a median follow-up of 17 months (mos), mOS was 28 mos (95% CI 23-NR). Considering most frequent mutations, mOS was 25 (95% CI 16-NR) and 54 mos (95% CI 29-NR) in pts with CDKN2A mutated and wild-type (WT) BTC (p = 0.061). mOS was 36 mos (95% CI 14-NR) in ARID1A mutated pts versus (vs) 28 mos (95% CI 16-NR) in WT ones (p = 0.931), 25 (95% CI 10-NR) vs 36 mos (95% CI 17-NR, p = 0.069) in TP53 mutated vs WT pts, and 17 (95% CI 10-NR) vs 29 mos (95% CI 25-NR) in KRAS mutated and WT pts, respectively (p = 0.068). As far as druggable mutations are concerned, mOS was 41 (95% CI 25-NR) vs 29 mos (95% CI 16-NR) in IDH1 mutated and WT pts (p = 0.299), whereas FGFR2 rearranged pts had a mOS of 39 mos (95% CI 15-NR) vs 28 (95% CI 23-NR, p = 0.891). 9 pts (11%) received targeted therapy with ivosidenib for IDH1 mutation (2 pts), pemigatinib for FGFR2 rearrangement (1 pt), derazantinib for FGFR2 mutation (3 pts), zanidatamab for HER2 amplification (2 pts), encorafenib for BRAF mutation (1 pt): mOS was 39 mos (95% CI 28-NR) vs 26 (95% CI 16-NR, p = 0.551).

**Conclusion:** In our study, no molecular alteration showed a clear prognostic value, although pts with CDKN2A, TP53, and KRAS mutations had worse clinical outcomes. mOS was numerically longer in pts receiving targeted therapy, and in those with IDH1 mutated or FGFR2 rearranged tumors, but failed to reach statistical significance, probably due to the small sample size. Larger studies could provide additional information regarding the prognostic and predictive value of pivotal molecular alterations in BTC.

**Figure:**



# PO7-14-YI

# A multi-center comparison of Atezolizumab plus Bevacizumab and Lenvatinib as primary systemic therapy for unresectable hepatocellar carcinoma: focus on thrombotic and hemorragic adverse events

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Email: silvia.gaia74@gmail.com

**Background and aims:** First line therapy for unresectable hepatocellular carcinoma (HCC) are Atezolizumab plus Bevacizumab (AB) and Lenvatinib (Len). The aim is to compare their efficacy and safety, mainly focusing on thrombotic and hemorrhagic adverse events.

**Method:** A multi-center retrospective study was conducted. All patients treated with Len or AB for HCC in 3 northern Italian centers were consecutively enrolled. Demographic, clinical and radiologic data were collected. All patients were treated following EASL guideline for the HCC systemic therapy. Lev was available since March 2020 and AB since January 2022.

**Results:** Overall 173 patients receiving AB (n = 65) or Len (n = 108) were enrolled. At baseline the 2 groups were similar for sex (85% male), median age (69 years old), diagnosis of cirrhosis (81%) vs chronic liver disease (19%), Child Pugh A (100%), HCC staging (75% BCLC-C, 25% BCLC-B), presence of low risk of esophageal varices (39%), median platelet count (150 x$10^9$/L), AFP (median 24.5 ng/ml), and anticoagulant therapy (21%). Anti-aggregant therapy was more frequent in Lev group (27.8%) compared to AB (12.3%), p = 0.022. Viral aetiology of liver disease was higher in AB group (66.7%) vs Len goup (48.1%), (p = 0.050). Median follow-up was longer in Lev group (11.3 months, IQR 5.7-21.4) vs AB (6.8 months, 4.1-11.1), p <0.001. In Len group 6 patients underwent liver transplantation and 1 patient in AB group.

Mean overall survival (OS) was 18.8 months (95% CI 16.2-21.6), without difference between Len and AB group (p = 0.454) (Figure 1). OS was related to radiological response at 3 months (p <0.001) in both groups. Baseline AFP value was associated to higher mortality only in Len group, (p <0, 001).
Disease control rate at 3, 6 and 12 moths was similar in Lev group *vs* AB group: 77% *vs* 66%, 64% *vs* 56% and 48% *vs* 37%, respectively (p = ns).

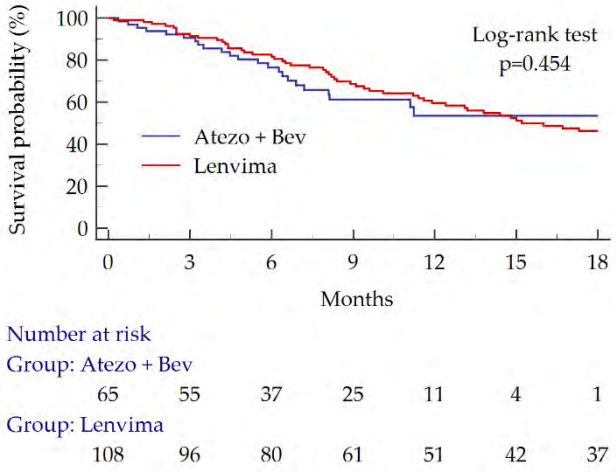
Thrombotic events were uncommon in both groups: 5/108 (4.6%) in Len group [2 acute coronary syndrome, 1 portal vein thrombosis (PVT), 1 Pulmonary embolism, 1 transitory ischemic attack] *vs* 3/65 (4.6%) in AB group (3 PVT) (p = 0.561). No thrombotic events occurred in patients with anticoagulants. Overall the use of anti-platelets drug did not reduce the risk of thrombotic events (HR = 1.69, p = 0.481) nor increased the risk of hemorrhagic events (HR = 1.08, p = 0.870).

Mild and/or severe hemorrhagic events occurred in 15/108 (13.9%) in Lev group and in 10/65 (15, 3%) in AB group (p = 0.421): the use of anticoagulant drugs was significantly associated to an increased risk of hemorrhagic events (HR 2.61, p = 0.02).

Hospitalization for any reason occurred in 1/108 patient treated with Len and in 20/65 patients treated with AB, 4 of them related to hemorragic events.

**Conclusion:** AB or Lev had comparable overall survival and disease control rate. In both group thrombotic events were uncommon: therapy with anticoagulants avoided the risk of thrombotic events but increased the hemorrhagic risks.

**Figure:**



# PO7-15
# Did we apply the BCLC classification correctly? Results from the III hepatocellular carcinoma registry of the Spanish Association for the Study of the Liver (AEEH)

Margarita Sala[1 2], Sonia Pascual[3], Rosa Rota[4], Ana M Matilla[5], Marta Campos[2 6], Manuel Delgado[7], MARIA TERESA FERRER RIOS[8], Jose Luis Montero[9], Xavier Segarra Ortega[10], Antonio Guerrero[2 11], Carlos Aracil[12], CARLOS RODRIGUEZ LOPE[13], Marta Romero-Gutiérrez[14], Miguel Sogbe[15], Sergio Vaquez Rodríguez[16], Javier Fuentes Olmo[17], Beatriz Minguez[2 18], Luis Cortés García[19], Esther Molina[20], Paloma Rendon[21], ARIADNA CLOS PARALS[22], Dácil Díaz Bethencourt[23], Araceli García-Sánchez[24], Raisa Quiñones[25], Francisco Javier Bustamante Schneider[26], Christie Perelló[27], Juan José Urquijo[28], HERNAN ANDREU SERRA[29], Camilo Julio Llamoza-Torres[30], Silvia Montoliu[31], Cristina Fernández Marcos[32], Ana Guiberteau[33], Manuel Hernandez-Guerra[34], Mercedes Vergara Gómez[35], Alexia Fernández López[36], María Paz Valer Lopez-Fando[37], Maria Luisa Gutierrez[38], Tania Hernáez-Alsina[39], Susana Coll[40], Berta Cuyas[41], María Julia Morillas Ariño[42], SUSANA REBOLLEDO OLMEDO[43], Miguel Fernandez-Bermejo[44], Mercé Roget[45], Irina Calvo[46], GEMMA PACHECO DEL RIO[47], Raimon Rifà Fornt[48], Pilar Conde[49], Monica Llorente Barrio[50], Mariano Gómez-Rubio[51], Irene Peñas Herrero[52], Maria Varela[53]

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**Background and aims:** Hepatocellular carcinoma (HCC) arises on cirrhotic livers in >80% of cases, so its staging considers tumour burden, symptoms associated with the ECOG Performance Status Scale (ECOG-PS) and liver function. The BCLC classification, the most widely used and globally recommended staging system for HCC, encompasses these three parameters along with the recommended treatment. The aim of this study was to analyse how the guidelines on BCLC classification and recommended treatments were adjusted among the participating centres of the III HCC Registry of the AEEH.

**Method:** Multicentre prospective data were collected from the III AEEH Registry including HCC patients diagnosed between 1-oct-22 and 31-jan-23. Data related to tumour staging and treatment were reviewed. Discrepancies and inconsistencies were checked with the sites to rule out mistakes.

**Results:** Data of 695 HCC patients (52 centres) was analysed. Concerning the BCLC classification, 54 patients (7.7 %) were misclassified. Specifically, in BCLC-0 patients (n = 85), 5 cases (5.8 %) should be reclassified as: BCLC-A (n = 3 due to tumour characteristics) and BCLC-D (n = 2 due to liver dysfunction with contraindication for liver transplantation (LT)). In BCLC-A patients (n = 304), 19 cases (6.2 %) should be reclassified as: BCLC-0 (n = 6 due to tumour size), BCLC-B (n = 3 due to tumour multinodularity out of Milan criteria), BCLC-C (n = 1 due to vascular invasion (V.I.), n = 6 due to ECOG PS-1) and BCLC-D (n = 3 due to liver dysfunction with contraindication for LT). In BCLC-B patients (n = 86), 20 cases (23.2 %) should be reclassified as: BCLC-A (n = 10 due to single tumours, n = 1 due to 3 nodules <3 cm), BCLC-C (n = 7 due to ECOG PS-1), and BCLC-D (n = 2 due to liver dysfunction with contraindication to LT). In BCLC-C patients (n = 142), 6 cases (4.2 %) should be reclassified as: BCLC-B (n = 1 due to absence of V.I extrahepatic spread (EH) and ECOG-PS >0) and BCLC-D (n = 5 due to ECOG PS-3 and/or liver dysfunction). In BCLC-D patients (n = 78), 4 cases (5.1%) were reclassified as: BCLC-A (n = 1 due to Child-Pugh C but indication for LT), and BCLC-C (n = 3 due to absence of liver dysfunction or ECOG-PS >2).In 9/695 (1.29%) patients, the assigned treatment didn't follow guideline recommendations. Specifically, surgical resection was performed in 3 patients with V.I or EH, ablation in 2 patients with EH and Child C with contraindication for LT, chemoembolization in 2 with V.I, radioembolization in 1 with EH, and fractionated stereotactic body radiotherapy in 1 with EH. To note, all these decisions were collegiate choices within local multidisciplinary committees.

**Conclusion:** Overall, the Spanish centres participating in the III AEEH Registry have a remarkable adherence to the guidelines, given that more than 92% of the patients were well classified according to the BCLC, and more than 98.5% received a first treatment following the guidelines.

# PO7-16
# Exploring self-reported occupational exposures in patients with MASLD-related hepatocellular carcinoma and/or cirrhosis: a prospective pilot study

Francesco Tovoli[1], Bernardo Stefanini[1], Daniele Mandrioli[2], Stefano Mattioli[3], Andrea Vornoli[2], Daria Sgargi[2], Fabiana Manservisi[2], Fabio Piscaglia[1], Stefania Curti[1], Luigi Bolondi[1]

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**Background and aims:** Metabolic dysfunction-associated steatotic liver disease (MASLD) has been traditionally associated with insulin resistance and obesity. Recently, pollutants have been shown to contribute to the development of MASLD. Given the global burden of MASLD, understanding whether pollutants are merely associated with steatosis or contribute to its progression to advanced chronic liver disease (ACLD) and hepatocellular carcinoma (HCC) is critical. Workers exposed to occupational toxicants represent an ideal population for assessing the potentially hazardous consequences of professional exposure. Confirming a link between occupational exposure and ACLD/HCC may not only provide further elements in understanding MASLD, but also contribute to preventive strategies for exposed workers.This study aimed to assess the prevalence of self-reported occupational exposure to toxicants in patients with MASLD.

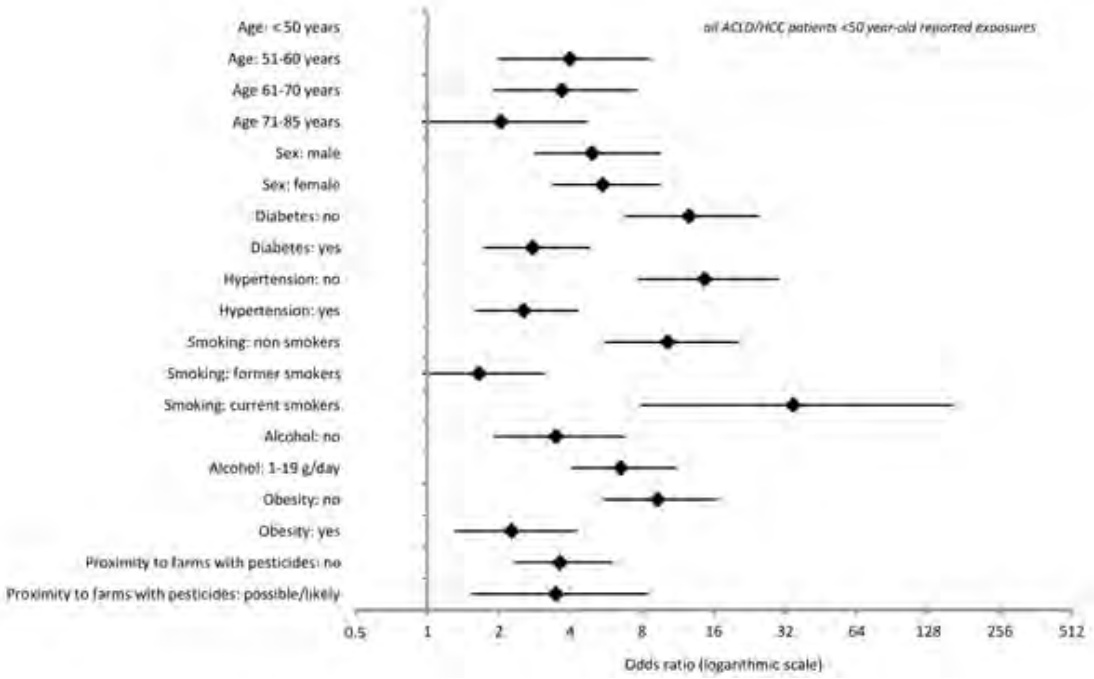
**Method:** This hospital-based prospective pilot study included 201 patients with MASLD. Data on workplace toxicant exposure were collected systematically using a structured questionnaire. Subsequently, patients with ACLD and/or HCC (n = 55) were compared to controls (n = 146). Logistic regression analysis and propensity score models were used to investigate the associations between self-reported occupational exposure and ACLD and/or HCC.

**Results:** Patients with ACLD/HCC reported exposure to metals, halogenated refrigerants, pain/resins, and fuel emissions more often than the controls. After controlling for confounders, durations of 21-30 years and >30 years of occupational exposure to toxicants showed odds ratios (ORs) of 2.31 (95% confidence interval [CI]: 1.09-4.88, p = 0.029) and 4.47 (95% CI: 2.57-7.78, p <0.001), respectively.

**Conclusion:** In this pilot study, patients with MASLD complications were more likely to report workplace toxicant exposure. Our results warrant future multicentre confirmatory studies, as implementing prevention policies may reduce the risk of life-threatening diseases among exposed populations.

**Figure:**

**Figure:** Odds ratios for advanced chronic liver disease and/or hepatocellular carcinoma in patients reporting exposure to workplace toxicants for >20 years.



# PO7-17
# Diagnostic performance of CT/MRI LI-RADS v2018 in non-cirrhotic steatotic liver disease

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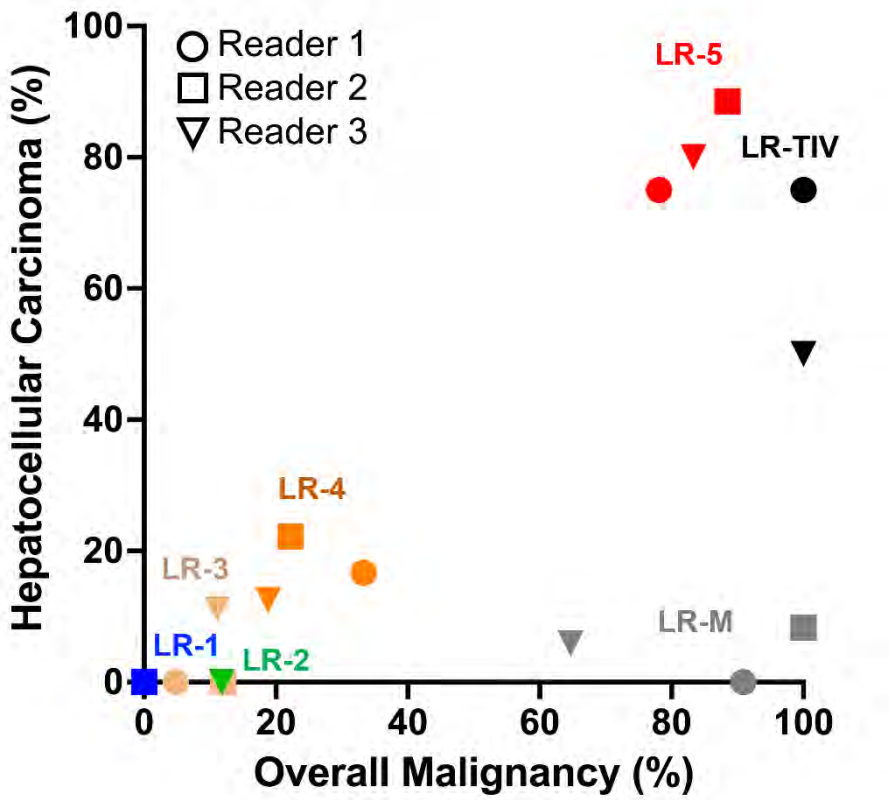
**Background and aims:** Steatotic liver disease (SLD) is rapidly becoming the primary driver of chronic liver disease and related complications, including hepatocellular carcinoma (HCC). A substantial proportion of patients develop HCC without cirrhosis. CT/MRI LI-RADS v2018 (LI-RADS) has not yet been studied in this patient population. The objective of our study was to assess the performance of LI-RADS among patients with non-cirrhotic SLD.

**Method:** This IRB-approved, retrospective study included 119 observations from 77 adult patients (36 women, 41 men; median age 64 years) who received liver protocol CT or MRI from 2010 to 2023. All patients had histopathologic evidence of SLD but no cirrhosis. Three board-certified abdominal radiologists blinded to the final tissue diagnosis assessed each observation per LI-RADS and assigned a final category. Inter-reader agreement with weighted kappa was calculated for major features and final category. The positive predictive value, sensitivity, specificity, and accuracy in identifying HCC and overall malignancy was calculated.

**Results:** 75 observations (63%) were benign and 44 (37%) were malignant. Positive predictive value for HCC was 0-0% for LR-1, 0-0% for LR-2, 0-7% for LR-3, 11-20% for LR-4, 75-88% for LR-5, 0-8% for LR-M, and 50-75% for LR-TIV. For malignancy (including HCC), positive predictive value was 0-0% for LR-1, 0-11% for LR-2, 3-9% for LR-3, 16-31% for LR-4, 78-88% for LR-5, 65-100% for LR-M, and 100-100% for LR-TIV. For LR-5 in identifying HCC, sensitivity was 79-83%, specificity was 91-97%, and accuracy was 89-92%. For composite categories of LR-5, LR-M, or LR-TIV in identifying overall malignancy, sensitivity was 86-89%, specificity was 85-96%, and accuracy was 86-93%. Most common false positives for LR-5 were hepatocellular adenomas. Inter-reader agreement for final category was 0.766.

**Conclusion:** LI-RADS 5 still most commonly represents HCC, but its specificity is lowered due to misclassification of hepatic adenomas.

**Figure:**


Reader 1
Reader 2
Reader 3
LR-5
LR-TIV
LR-4
LR-3
LR-M
LR-1
LR-2
Hepatocellular Carcinoma (%)
Overall Malignancy (%)
0
20
40
60
80
100

# PO7-18
# Population pharmacokinetic modeling of orally administered fostroxacitabine bralpamide (fostrox, MIV-818) and its metabolite troxacitabine in a phase I/IIa liver cancer study

Karin Tunblad[1], Pia Baumann[1], Sujata Bhoi[1], Hong Jae Chon[2], T.R. Jeffry Evans[3], Jeong Heo[4], Malene Jensen[1], Ruth Plummer[5], María Reig[6], Debashis Sarker[7], Hans Wallberg[1], Fredrik Oberg[1], Lars Lindbom[8]

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**Background and aims:** Fostrox is an orally administered troxacitabine-based nucleotide prodrug that is designed to direct high levels of the active metabolite to the liver through first pass metabolism. Fostrox is in clinical development in combination with lenvatinib in patients with hepatocellular carcinoma (HCC). Here we present preliminary results from population pharmacokinetic (PK) modeling of data from the first clinical study with fostrox (NCT03781934).

**Method:** Fostrox and troxacitabine plasma concentration-time data from patients treated with fostrox (3-70 mg for up to 5 consecutive days in 21-day cycles) monotherapy or in combination with lenvatinib (standard dose) were analyzed using nonlinear mixed effects modeling (NONMEM 7.2). PK samples were collected pre-dose and up to 15 days after the first dose of fostrox in a cycle. One-, two- and three-compartment disposition with first/zero-order input models and linear/nonlinear elimination were tested. Clearance and volume parameters were scaled allometrically with standard scaling terms (0.75 and 1), challenged for the final model. Subsequently, effects of creatinine clearance (CRCL) and dose were exploratorily evaluated. The data for fostrox and troxacitabine had varying proportions of samples below the lower limit of quantification (58% and 12%, respectively). Importance Sampling Expectation Maximization (IMPMAP) estimation method was therefore used to allow for accurate and stable model estimation. Model goodness-of-fit (visual predictive check, bootstrap) was assessed.

**Results:** Forty-two patients from the phase I/IIa clinical study contributed 834 observations each for fostrox and troxacitabine. Forty-seven percent of the patients had normal renal function (CRCL ≥90 ml/min), while 43% and 10% had mild (CRCL 60-87 ml/min) and moderate (CRCL 48-59 ml/min) renal impairment, respectively. Fostrox data were described by a 1-compartment PK model parameterized in terms of systemic oral clearance (CL/F), central volume of distribution (Vc/F), serial zero-order absorption duration (D) and first-order absorption rate constant ($k_a$). Between-subject variability (BSV) was estimated for D, CL/F and relative bioavailability.Troxacitabine data were described by a 2-compartment PK model parameterized in terms of CL/F, intercompartmental clearance (Q/F), Vc/F and peripheral volumes of distribution (Vp/F), lagged D and $k_a$. BSV was estimated for D, $V_C$/F and relative bioavailability.

**Conclusion:** The PK models described the fostrox and troxacitabine data well with dose linear increase in exposure and linear elimination of both moieties.

# PO8-01
# Efficacy and safety of atezolizumab-bevacizumab for hepatocellular carcinoma in real-life clinical practice: data from a multicenter collaborative study

Francesco Tovoli[1], Caterina Vivaldi[2], Piera Federico[3], Bernardo Stefanini[1], Andrea Palloni[4], Caterina Soldà[5], Andrea Dalbeni[6], Lorenzo Lani[1], Ingrid Garajovà[7], Stefano Tamberi[8], Stefania De Lorenzo[9], Alessandro Granito[1], Dante Pio Pallotta[1], Gianluca Masi[2], Sara Lonardi[5], Giovanni Brandi[1], Bruno Daniele[3], David Sacerdoti[6], Benedetta Stefanini[1], Gianluca Svegliati-Baroni[10], Claudia Campani[11], Elisa Pellegrini[11], Fabio Piscaglia[1]

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**Background and aims:** Atezolizumab/bevacizumab (AB) is the current standard-of-care for patients with unresectable hepatocellular carcinoma. Most of efficacy and safety data derive from clinical trials, while only few real-life clinical practice studies have been published. We aimed at providing To provide real-life clinical data of HCC patients treated with AB.

**Method:** The ARTE study group prospectively collects data of patients who started AB outside of clinical trials. We evaluated clinical data and outcome of HCC patients included in the ARTE database (March 2022-November 2023).

**Results:** Data of 157 patients from 12 centres were collected. Most patients had advanced HCC (59.9%).Twenty-seven (17.1%) patients had ≥1 condition (s) outside of the IMbrave-150 enrolling criteria (thrombocytopenia<70.000/mmc [n = 8], concurrent/recent neoplasia [n = 6], concurrent anticoagulation [n = 6], arrhythmia [n = 5], HIV infection [n = 4], chronic heart failure [n = 2]). HCV was the most commonly reported etiology (43.9%), followed by MASLD (31.8%), ALD (23.6%), and HBV (14.0%). Forty-four (28%) patients reported multiple etiologies. The prevalence of performance status (PS)>0, macrovascular invasion (MVI), extrahepatic spread, and alpha-fetoprotein (AFP)>400 ng/ml was 38.2, 37.6, 38.2, and 29.9%, respectively. Nineteen (12.1%) patients received surgical (n = 3), percutaneous (n = 3), trans-arterial treatments (n = 4), or non-liver-directed radiotherapy (n = 9) after the start of AB. Median overall and progression-free survivals were 19.8 (95% CI 15.8-23.8) and 10.5 months (6.3-14.7), respectively. MVI, AFp >400 ng/ml, ALBI grade>1, and platelet-to-lymphocyte ratio>210 were independent negative prognostic factors. Progression due to new extrahepatic lesions/macrovascular invasion led to worse outcomes.The most common treatment-related adverse events (AEs) included fatigue (42.3%), hypertension (28.2%), anorexia (18.6%), and diarrhoea (17.2%). Most common treatment-related Grade 3-4 AEs were: hypertension (7.0%), digestive non-variceal bleeding (3.8%), increased aminotransferases (3.2%), and variceal bleeding (2.5%).

**Conclusion:** these real-life data confirm previous efficacy and safety information of AB. Multiple HCC etiologies, comorbidities, and combination with locoregional treatments are common in clinical practice and warrant dedicated studies.

# PO8-02
# Readability, reliability, and accuracy of publicly available large language models on liver cancer diagnosis and management

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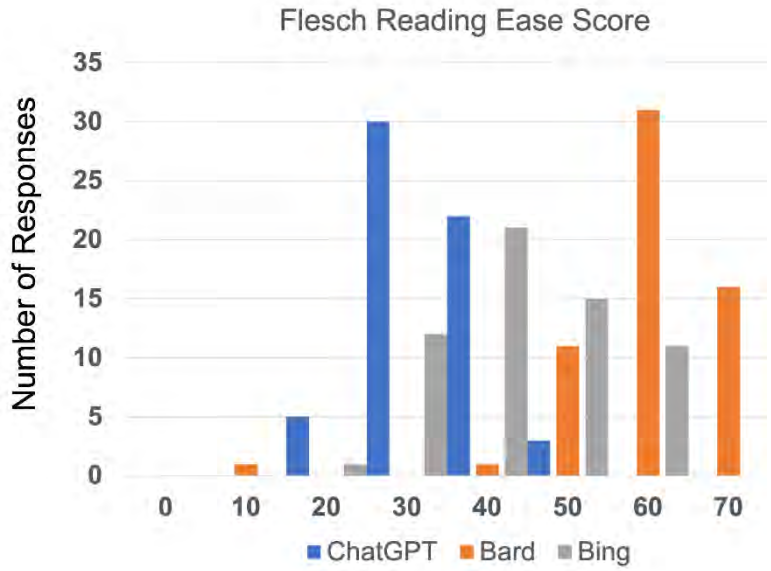
**Background and aims:** To assess the readability, accuracy, and reliability of publicly available large language models (LLMs) in answering fundamental questions on liver cancer (hepatocellular carcinoma) diagnosis and management.

**Method:** Twenty questions on liver cancer diagnosis and management were asked in triplicate to ChatGPT-3.5 (OpenAI), Bard (Google), and Bing (Microsoft) in August 2023. Responses were quantified for readability using the Flesch-Kincaid test, ranging from 0 (professional level, i.e. extremely difficult to read) to 100 (5th grade, i.e. very easy to read). Responses were also assessed by a panel of fellowship-trained physicians from three academic liver transplant centers who actively diagnose and/or treat liver cancer: two abdominal radiologists, two interventional radiologists, one medical oncologist, and one hepatologist. Responses were categorized as accurate (score 1; all information is true and relevant), inadequate (score 0; all information is true, but either does not fully answer the question or irrelevant information is provided), or inaccurate (score -1; any information is false). Means with standard deviations were recorded. Responses were considered as a whole accurate if mean score was >0 and reliable if mean score was >0 across all responses for the single question.

**Results:** ChatGPT responses were the least readable (mean 29; college graduate; range 13-49), followed by Bing (40; college; range 19-59), and then Bard (55; 10th-12th grade; range 6-68; $p < 0.001$). Comparatively, the twenty questions' mean readability was 77 (7th grade; range 57 to 89; e.g. "What is liver cancer?"). Out of 60 responses, ChatGPT, Bing, and Bard had 27 (45%), 32 (53%), and 16 (27%) that were considered accurate. Each LLM was only able provide reliable responses for 5/20 (25%) of questions. All LLMs confused US LI-RADS categories for those of CT/MRI LI-RADS. All LLMs had responses that recommended a biopsy for a LR-5 observation to confirm hepatocellular carcinoma. All LLMs had difficulties with LR-TIV and LR-M, either not recognizing them altogether or confusing them for other categories, e.g. presenting LR-TIV as a treated observation.

**Conclusion:** Large language models provide complex responses to relatively simple questions on liver cancer diagnosis and management that are seldomly accurate or reliable.

**Figure:**


Flesch Reading Ease Score
Number of Responses
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
ChatGPT
Bard
Bing

# PO8-04
# Establishment of a national hepatocellular carcinoma service has addressed regional inequality in access to treatments including liver transplant

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**Background and aims:** St. Vincent's University Hospital (SVUH) is home to the national liver transplant unit. A dedicated hepatocellular carcinoma (HCC) service was established in 2014 and is the '*de facto*' national HCC centre. SVUH is the only hospital in Ireland to offer full array of approved treatment modalities for HCC. Our study looks at the BCLC (Barcelona Clinic Liver Cancer) stage of diagnosis based on the referral county origin, (within Dublin versus outside Dublin), over 2 time periods, 2014-2016 (during service development) and 2017-2022 (while service is established). In addition, we looked at how this affected curative treatment rates with liver transplantation (LT).

**Method:** All patients referred from 2014 to 2022 were included in the study. They were divided into 2 time periods (1) 2014-2016 and (2) 2017-2022, then sub-divided by referral county; Dublin or Outside Dublin. The patient demographics were obtained from a prospectively maintained clinical HCC database.

**Results:** The dedicated service has grown year on year with a 90% increase in confirmed HCC diagnoses between 2014 and 2022. 978 patients were included in this study. 242 (25%) in time period 1 (2014-2016) and 736 (75%) in time period 2 (2017-2022).

| **Dublin** | | | **Outside Dublin** | | |
|---|---|---|---|---|---|
| BCLC Stage | 2014-2016 N = 157 (65%) | 2017-2022 N = 252 (34%) | BCLC Stage | 2014-2016 N = 85 (35%) | 2017-2022 N = 484 (65%) |
| **0/A** | **60%** | **43%** | **0/A** | **32%** | **38%** |
| B | 17% | 17% | B | 19% | 22% |
| C | 7% | 18% | C | 18% | 18% |
| D | 16% | 22% | D | 32% | 22% |

*Table 1: The percentage distribution of referrals based on the BCLC stage at diagnosis for the Dublin and Outside Dublin groups.*

During the 2 timeframes, the percentage of liver transplant recipients for HCC located outside Dublin has also increased significantly in the 2017-2022 cohort (from 15% to 59%).

**Conclusion:** In the initial years of the service, we noted marked disparity in the BCLC stage at referral from outside Dublin. Especially as only one -third of referrals were coming from the vast majority (75%) of the population. This inequity has significantly reduced since the HCC service inception and the establishment of robust referral pathways. Access to liver transplantation as a curative treatment option has also improved nationally.

# PO8-05
# Demographic and clinical features of the patients diagnosed with hepatocellular carcinoma in Spain: results of the III registry of the Spanish Association for the Study of the Liver (AEEH)

Margarita Sala[1 2], Sonia Pascual[3], Rosa Rota[4], Ana M Matilla[5], Marta Campos[2 6], Manuel Delgado[7], MARIA TERESA FERRER RIOS[8], Jose Luis Montero[9], Jesús Manuel González[2 10], Antonio Guerrero[2 11], Carlos Aracil[12], CARLOS RODRIGUEZ LOPE[13], Marta Romero-Gutiérrez[14], Miguel Sogbe[15], Sergio Vaquez Rodríguez[16], Javier Fuentes Olmo[17], Beatriz Minguez[2 18], Luis Cortés García[19], Esther Molina[20], Paloma Rendon[21], ARIADNA CLOS PARALS[22], Dácil Díaz Bethencourt[23], Araceli García-Sánchez[24], Raisa Quiñones[25], Francisco Javier Bustamante Schneider[26], Christie Perelló[27], Juan José Urquijo[28], HERNAN ANDREU SERRA[29], Camilo Julio Llamoza-Torres[30], Silvia Montoliu[31], Cristina Fernández Marcos[32], Ana Guiberteau[33], Manuel Hernandez-Guerra[34], Mercedes Vergara Gómez[35], Alexia Fernández López[36], María Paz Valer Lopez-Fando[37], Maria Luisa Gutierrez[38], Tania Hernáez-Alsina[39], Susana Coll[40], Berta Cuyas[41], María Julia Morillas Ariño[42], SUSANA REBOLLEDO OLMEDO[43], Miguel Fernandez-Bermejo[44], Mercé Roget[45], Irina Calvo[46], GEMMA PACHECO DEL RIO[47], Raimon Rifà Fornt[48], Pilar Conde[49], Monica Llorente Barrio[50], Mariano Gómez-Rubio[51], Irene Peñas Herrero[52], Maria Varela[53]

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**Background and aims:** Epidemiology of hepatocellular carcinoma (HCC) is changing. The aim of this study is to carry out a third national registry of patients diagnosed with HCC in Spain and compare patient's features with those of the previous registries of the AEEH.

**Method:** Prospective multicenter collection of demographic and clinical data of patients diagnosed with primary liver cancer between 1-Oct-22 and 31-Jan-23. Descriptive and comparative analysis with data recorded by the same centers in previous I (2008) and II (2014) AEEH's Registries.

**Results:** A total of 767 patients (52 centers) were included in the III Registry, 91 % with HCC. HCC patients were mainly male (83.3 %) with a median age of 68 years, having cirrhosis (80.7 %). The most frequents etiological factors were alcohol associated, contributing alone (29.9%) or with other factors (54.9 %), chronic hepatitis C infection (HCV, 17.3 %) and metabolic dysfunction-associated steatotic liver disease (MASLD, 10.5 %). Treatments for HCC were ablation (n = 109, 15.7 %), systemic (n = 102, 14.7 %), chemoembolization (n = 101, 14.6 %), evaluation for liver transplantation (n = 90, 13 %), resection (n = 86, 12.4 %), radioembolization (n = 33, 4.8 %), and stereotactic body radiotherapy (SBRT) (n = 4, 0.6 %). For comparison between the three registries, data from 29 centers were analyzed (n = 1351; n = 432 from I, n = 427 from II, n = 492 from III). A significant progressive increase of MASLD (1.9 % vs 5.9 % vs 11.8 %) and HCC in non-cirrhotic liver (4.2 % vs 3.8 % vs 7.9 %) as well as a decrease in HCV (43 % vs 28.7 % vs 17.5 %) ($p < 0.0001$) were observed. No changes in the alcohol etiology were found (29.8 % vs 34.7 % vs 29.7 %). In addition, there was a slight increase in the number of men ($p = 0.031$), prevalence of hypertension, diabetes and obesity ($p < 0.001$), with no differences in screening detection ($p = 0.398$) or liver function ($p = 0.178$).At present, HCC is still diagnosed outside the screening program in 43.9 % of patients with cirrhosis. The profile of patients was the same as in I and II Registries: high proportion of alcohol etiology, male gender, active alcohol and tobacco consumption. Diagnosis outside screening was associated with larger tumor size (44.5 vs 26 mm), extrahepatic spread (17.3 vs 2.9 %), vascular invasion (30.2 vs 11.7%) and advanced BCLC stage (45.2 % BCLC-C and D vs 17.6 % BCLC-C and D), $p < 0.0001$. Consequently, these patients received radical treatments less frequently (27.8 vs 55.3 %) and few of them were evaluated for LT (6.8 vs 20.1%) $p < 0.0001$.

**Conclusion:** The proportion of patients with HCC diagnosed within the screening program did not change. However, we found significant etiological changes including a decrease in HCV, an increase in MASLD, and HCC arising in non-cirrhotic livers. These results indicate that policies to increase screening and prevention strategies targeting patients with alcohol abuse consumption and MASLD should be implemented.

# PO8-06-YI
# Household income is associated with a higher likelihood of receiving curative treatment in hepatocellular carcinoma

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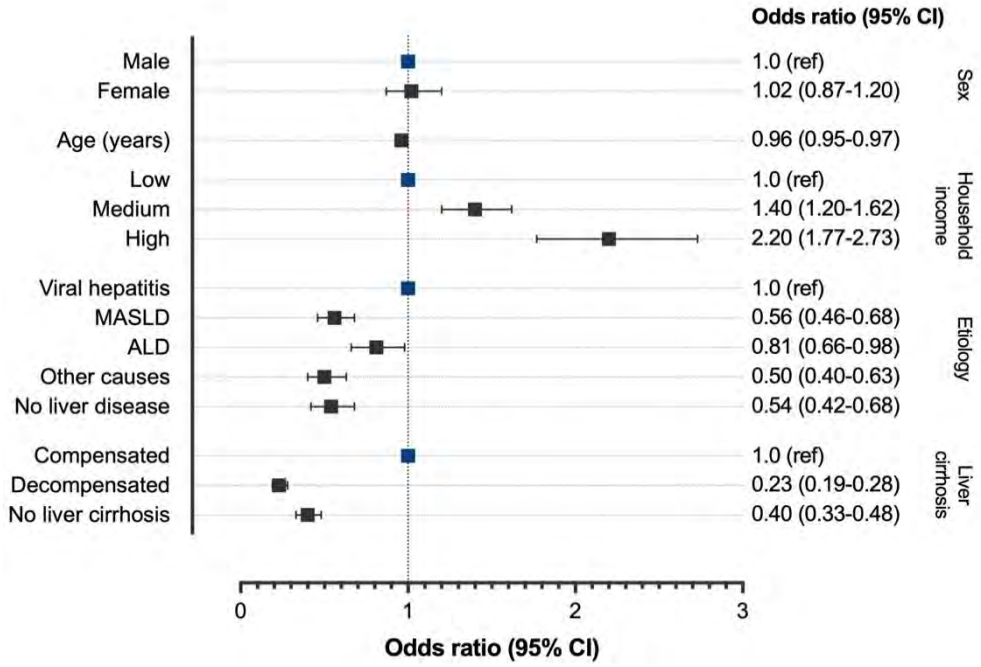
**Background and aims:** European studies examining the importance of socioeconomic status on outcomes in patients with hepatocellular carcinoma (HCC) are scarce. We aimed to examine whether household income was associated with receiving curative treatment in HCC.

**Method:** Using the Swedish quality register for liver cancer (SweLiv), we identified all adult patients diagnosed with HCC in Sweden (2012-2018). Baseline data were retrieved from SweLiv and other nationwide registers. Patients were stratified into three groups: low (poorest), medium (second and third quartile), and high household income (wealthiest); based on the distribution of all household incomes in Sweden. Logistic regression models were constructed to obtain odds ratios (OR) for receiving curative treatment (ablation, resection or liver transplantation). Clinically relevant variables were included in the adjusted model.

**Results:** Of 3473 patients with HCC, 1598 (46%), 1439 (41%), and 436 (13%) had low, medium, and high household incomes. The median age at diagnosis was 69 years and most were male (76%). A total of 1247 (36%) received treatment with curative intention: transplantation (n = 227), resection (n = 530), and ablation (n = 490). Household income was associated with increased likelihood of receiving curative treatment also after adjustment for all parameters in the BCLC staging system and other possible confounders such as disease etiology (**Fig. 1**). Compared to patients with low household income, the OR for curative treatment receipt for those with medium and high household income were 1.40 (95% Confidence Interval [CI] = 1.20-1.62) and 2.20 (95%CI = 1.77-2.73), respectively (**Fig. 1**). In the fully adjusted model, medium and high household income were still associated with higher likelihood of curative treatment, although to a somewhat lesser extent: aOR 1.31 (95%CI = 1.03-1.68) and aOR 1.94 (95%CI = 1.36-2.78), respectively.

**Conclusion:** Higher household income was associated with higher likelihood of receiving curative treatment in HCC. More efforts are needed to counteract the negative impact of health inequity in the management of patients with HCC.

**Figure:**



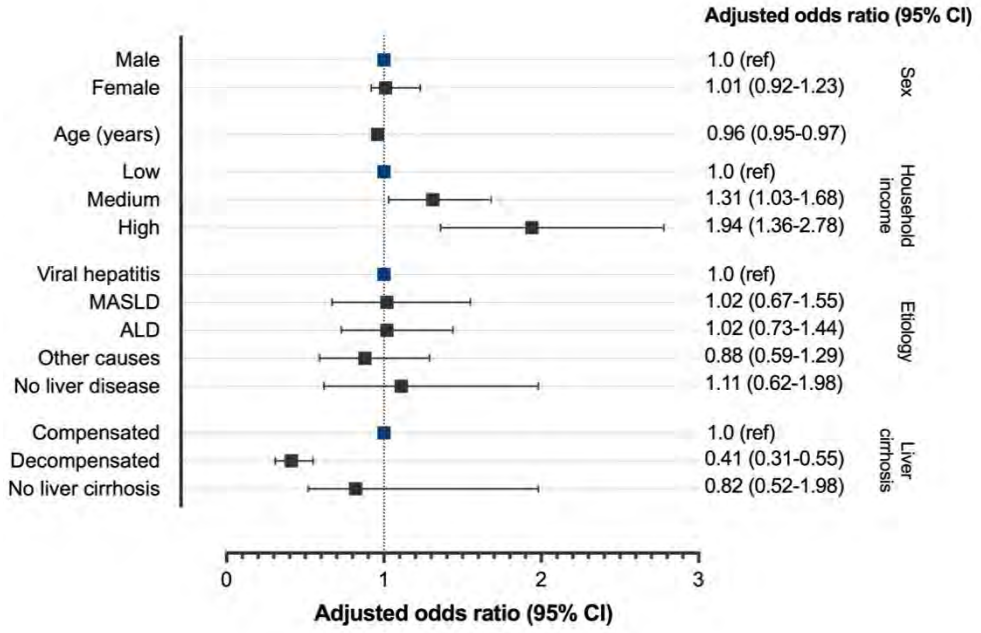




**Fig. 1.** Likelihood of curative treatment receipt in hepatocellular carcinoma in Sweden. Treatments with curative intention: transplantation, resection, and ablation. The multivariable model included all variables with adjusted odds ratios (ORs) shown plus: year of diagnosis (2012-2018), Eastern Cooperative Oncology Group performance status (0, 1, ≥2), tumor size in mm (<20, 20-30, >30), number of tumors (1, 2-3, >3), tumor thrombosis or portal vein thrombosis (yes/no), regional metastasis (yes/no), extrahepatic metastasis (yes/no), and comorbidity (arterial hypertension, type 2 diabetes, coronary artery disease). ALD: alcohol-related liver disease; CI: Confidence interval; MASLD: metabolic dysfunction associated steatotic liver disease.

# PO8-07
# Dosimetry and survival in unresectable primary hepatocellular carcinoma patients undergoing selective internal radiotherapy with resin 90Y: the interim analysis of a randomized clinical trial

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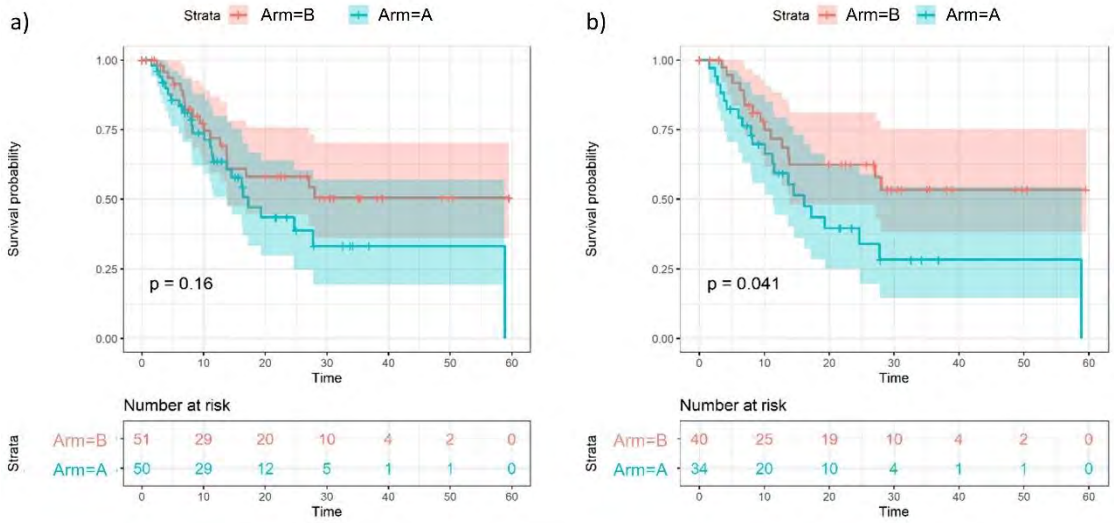
**Background and aims:** Selective internal radiotherapy (SIRT) is a radionuclide therapy for unresectable primary hepatocellular carcinoma (HCC) in which $^{90}$Y-loaded micron-sized embolic particles are directly injected into the hepatic arteries. The injection activity can be determined with body-surface-area (BSA) and MIRD mono-compartmental methods (both referred to as standard approach) or voxel-based dosimetry, based on a pre-treatment injection of $^{99m}$Tc-macro-aggregated-albumin used as a surrogate of the microspheres' distribution. The aim of this work is to perform a randomized clinical study to evaluate the impact of the two dosimetry approaches in terms of overall survival (OS).

**Method:** Patients with HCC diagnosis and treated with 90Y-loaded resin microspheres at two Institutes were randomized into arm A (activity prescription based on standard approach) or arm B (activity prescription based on novel voxel-based dosimetry approach) with dose constraints based on ref. (1, 2). The impact of the dosimetry approach in terms of patients' overall survival (OS) was investigated with Kaplan-Meier analysis implemented in R software. The patients' overall survival (OS) analysis for the two dosimetry approaches was investigated with Kaplan-Meier analysis with Log-rank test implemented in R software. The planned enrolment was of 150 HCC patients.

**Results:** Out of 101 treatments on enrolled patients, 75 and 26 were performed on males and females, respectively. Mean (range) age was 67 years (26-97). Median (range) follow-up was 16.3 months (1-59.5). Patients were divided between arm A (50) and B (51) for which the mean (range) administered activity was 1.54 (0.80-2.90) and 1.32 (0.79-3.29) GBq, respectively. The OS ratio at 12 months was 63.6% and 71.9% for arm A and B, respectively, while at 24 months was 43.5% and 58.2%, respectively. Kaplan-Meier analysis showed no statistical differences ($p = 0.16$, see Figure panel a). When the analysis was restricted to patients in which the prescribed activity differed by more than 5% between the two methods (i.e., 74 remaining patients), the OS in arm B was statistically significantly higher with respect to Arm A ($p = 0.041$, see Figure panel b). The OS ratio at 12 months was 59.3% and 71.8% in arm A and B, respectively, and 39.5% and 62.4% at 24 months, respectively.

**Conclusion:** The activity prescription based on pre-treatment voxel-based dosimetry showed a statistically significant superiority in OS compared to the standard dosimetry approach when the prescribed activity differed between the two approaches. References: (1)doi: 10.1007/s00259014-2824-5; (2)doi: 10.2967/jnumed.110.075861.

**Figure:**


a)
Strata Arm=B Arm=A
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.16
0 10 20 30 40 50 60
Time
Number at risk
Strata
Arm=B 51 29 20 10 4 2 0
Arm=A 50 29 12 5 1 1 0
0 10 20 30 40 50 60
Time
b)
Strata Arm=B Arm=A
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.041
0 10 20 30 40 50 60
Time
Number at risk
Strata
Arm=B 40 25 19 10 4 2 0
Arm=A 34 20 10 4 1 1 0
0 10 20 30 40 50 60
Time

# PO8-08
# Atezolizumab plus Bevacisumab versus lenvatinib for BCLC-B stage patients with hepatocellular carcinoma : a large real life worldwide population

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**Methods:** The study population included patients enrolled affected by intermediate (BCLC-B) HCC patients not suitable for locoregional therapies from eastern and western populations, who received A+B or Lenvatinib as first-line treatment. Univariate and multivariate analyses were used to evaluate predictor factors for overall survivor (OS) and progression free survivor (PFS) while prognostic factors were analyzed by univariate and multivariate analysis using Cox regression model.

**Results:** 919 BCLC-B HCC patients were enrolled in the study: 561 (61%) received Lenvatinib and 358 (39%) received A+B. The mOS for patients receiving Lenvatinib was 21, 3 months compared to 15, 8 months for patients receiving A+B as first-line treatment (Lenvatinib Vs A+B): HR 0, 84 p = 0, 22.
The mPFS for patients receiving Lenvatinib was 7, 34 months compared to 8, 68 months for patients receiving A+B as first-line treatment (Lenvatinib vs A+B): HR 1, 15 p = 0, 10.The multivariate analysis confirmed no different in terms of mOS and mPFS between the two treatments. Objective response rate (ORR) was 47, 11 % for patients receiving Lenvatinib and 27 % for patients receiving A+B p <0, 000001.Patients receiving Lenvatinib experienced a significantly higher incidence of hand-foot skin reaction (HFSR), hypertension, diarrhea, fatigue, decrease appetite, hypothyroidism, and other toxicity compared to patients receiving A+B. Favorable prognostic factors for OS in Lenvatinib group was platelets >100, HR 0, 68 p = 0, 02. No favorable prognostic factors were found for A+B group. Favorable prognostic factors for pfs in the A+B group were in those who performed at least one TACE previously HR 0.76 p = 0.02, platelets <100 HR 0.62 p = 0.0067, and nlr <3 HR 0.78 p = 0.04.

**Conclusion:** Although Lenvatinib had a greater response, the study showed no statistically significant differences between Lenvatinib and A+B in terms of efficacy, in these two cohorts of BCLC-B HCC patients.

# PO8-09
# Impact of hospital stay on complications and long-term outcomes after major hepatectomies

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**Background and aims:** Prolonged hospital stay following major abdominal surgery is often associated with worse outcomes. Liver is central to a multitude of functions within the body and hepatobiliary surgery is associated with significant morbidity. The primary aim was to examine the length of hospital stay after major hepatectomy in association with postoperative complications and readmissions.

**Method:** Data from 312 patients who underwent major hepatectomy at Heidelberg University Hospital between January 2017 and December 2021 were analyzed. Complications and readmissions of patients, grouped based on the empirical expectation of the hospital stay of up to 10 days (Group 1) and over 10 days (Group 2), were compared. Regression models were used to identify risk factors and predictors for readmissions.

**Results:** 213 patients had a prolonged hospital stay (Group 2). 59.6% in Group 2 developed major complications (Clavien-Dindo ≥3), while in Group 1, only 6% did (p = <0.001). PHLF and bile leaks occurred significantly more frequently in Group 2 (p = <0.001). Readmission was more frequent in Group 2 (70 cases, 32.9%) compared to Group 1 (14 cases, 14.1%) (p = 0.001). Kaplan-Meier analysis showed a significant difference in the readmission rates between the two groups, with dispersion 5 days after discharge. The most common cause for readmissions were infections (27.3%) and bile leaks (17.9%). Predictors for readmissions were major complications (OR: 3.395; 95% CI: 2.012-5.728; p = <0.001), PHLF (OR: 2.057; 95% CI: 1.237-3.419; p = 0.005), bile leakage (OR: 6.811; 95% CI: 3.803-12.196; P:<0.001), and a hospital stay of more than 10 days (OR: 2.972; 95% CI: 1.577-5.6; p = 0.001). The presence of a postoperative bile leak was identified as an independent predictor (OR: 3.051; 95% CI: 1.144-8.139; p = 0.026). The 5-year overall survival rate was 84.4% in Group1, compared to 77.5% in Group 2 (p = 0.238).

**Conclusion:** Patients with prolonged hospital stay are at an increased risk for readmission. Multicentral studies should assess the cut-offs and complication profiles to validate these findings. Further studies should investigate whether patients at risk for unplanned readmission would benefit from a scheduled follow-up at the discharging hospital shortly after discharge, e.g. after 5 days, to screen for potential complication. While 5-year overall survival wasn't statistically different between group, studies with increased sample size are needed to further elucidate how readmission after major hepatectomy affects survival.

**Figure**

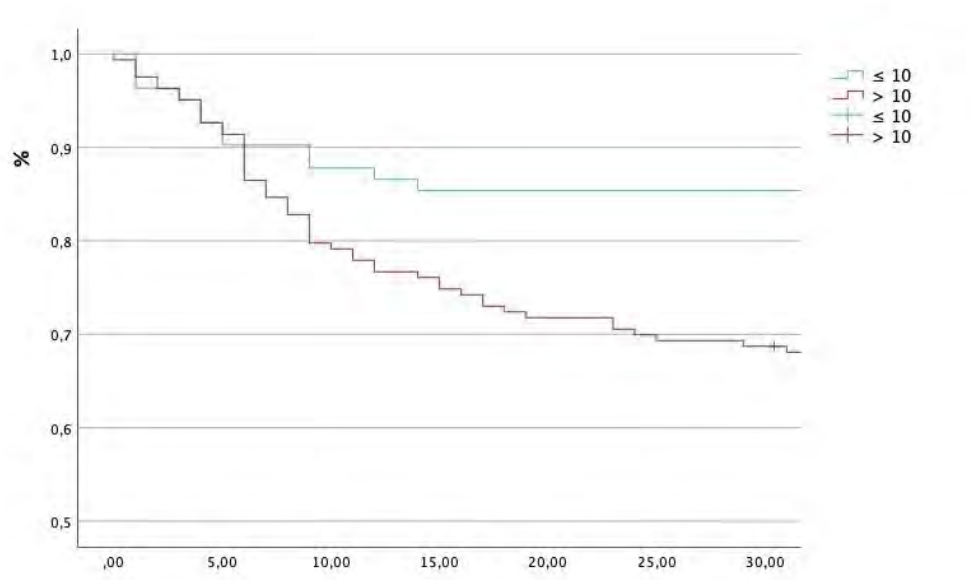


Figure 1 Kaplan-Meier Curve of Readmissions Within 30 Days

# PO8-10
# Thrombocytopenia did not increase radiofrequency ablation- related bleeding risk in patients with hepatocellular carcinoma and cirrhosis

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**Background and aims:** This study aimed to assess the bleeding risk during the perioperative period of radiofrequency ablation (RFA) in patients with hepatocellular carcinoma (HCC) and moderate thrombocytopenia.

**Method:** A retrospective analysis was conducted on 184 patients with cirrhosis and HCC who underwent RFA at the Department of Gastroenterology and Hepatology, West China Hospital, between June 2020 and February 2023. The patients were divided into two groups: group A (platelet count <50×109/L) and group B (platelet count ⩾ 50 × 109/L). The study compared the differences in postoperative complications and mortality within 90 days between the two groups. Additionally, regression analysis was performed to investigate the relationship between platelet count and postoperative complications.

**Results:** Group A comprised 58 patients with a median platelet count of 35×109/L, while Group B comprised patients with a median platelet count of 81×109/L. Group A had higher median INR values and incidence of ascites compared to Group B (1.3 vs. 1.2, p <0.01; 46.5% vs. 30.1%, p = 0.04). There were no significant differences in terms of BCLC stage, Child-Pugh grade, or prior decompensation events of cirrhosis between the two groups. Out of the total patients, 18 (9.8%) experienced postoperative complications, with 4 patients (2.2%) suffering from major perioperative bleeding. The incidence of major perioperative bleeding and postoperative complications did not significantly differ between group A and group B (0% vs. 3.2%, p = 0.31; 10.3% vs. 9.5%, p = 0.86). Regression analysis indicated that thrombocytopenia does not increase the risk of postoperative bleeding, while mild ascites was identified as an independent risk factor for postoperative complications. Within 90 days after the operation, three patients (1.6%) died, with only one patient's death attributed to operation-related causes.

**Conclusion:** This study suggests that RFA can be safely performed in patients with liver cirrhosis complicated by moderate thrombocytopenia. A preoperative platelet count of less than 50×109/L should not be considered a contraindication for RFA.

# PO8-13-YI
# Biliary tract cancers (BTC): molecular profiling and matched therapy

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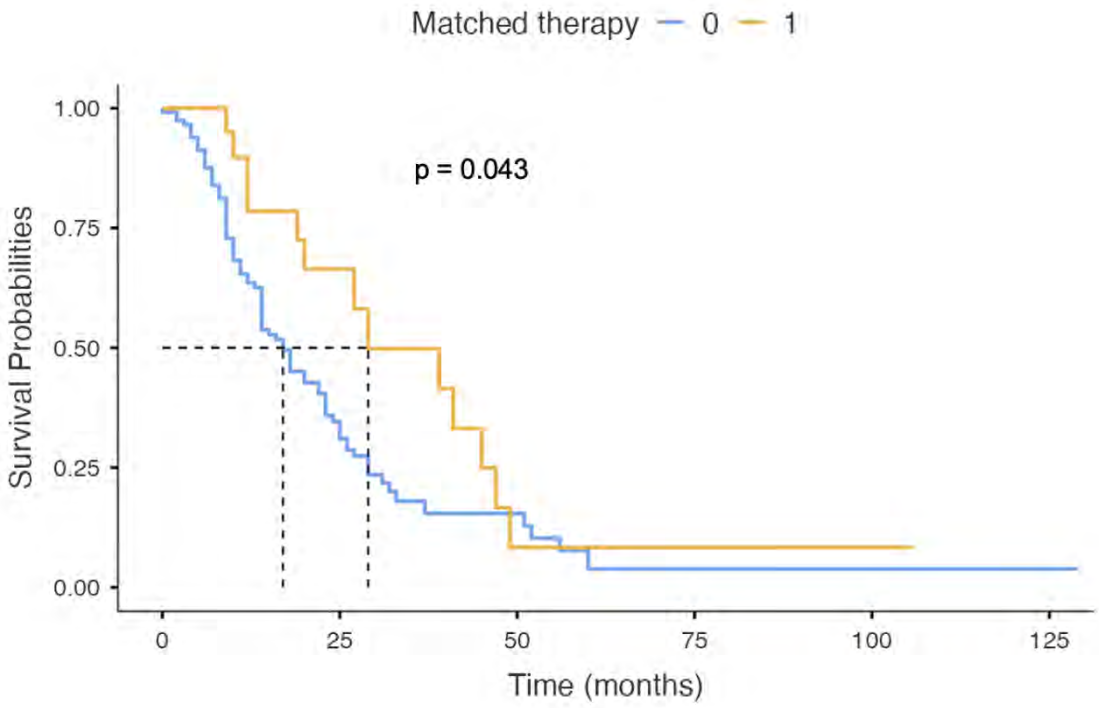
**Background and aims:** BTC are a group of genetically heterogeneous and aggressive diseases. Different molecular alterations have been identified and shown a prognostic and/or predictive value, allowing patients to receive targeted treatment based on specific mutations. Our aim was to identify the impact of systemic treatment (matched versus non-matched therapy) on clinical outcomes.

**Method:** The study population included patients with advanced BTC, available molecular profiling by single test or Next Generation Sequencing (NGS), and receiving at least one line of systemic treatment, evaluated at IRCCS Humanitas Research Hospital. The primary outcome was the impact of matched therapy on overall survival (OS) compared to patients receiving non-matched therapy. Median OS (mOS) was calculated from the start of first-line systemic treatment to date of death or last follow-up. Survival was calculated using Kaplan-Meier method and log-rank tests.

**Results:** 139 patients were included, with a median age of 62 years at diagnosis. 54% were male and 92% had intrahepatic cholangiocarcinoma (iCCA). 58% were diagnosed with metastatic disease and 88% received standard of care treatment. 68% were treated with a cisplatin-based first-line chemotherapy. 78% and 23% received systemic treatment in second- and further-line, respectively. 66 patients received single-test analysis, whereas 73 patients underwent NGS profiling on available tumor tissue. The most frequent mutations were CDKN2A, ARID1A and IDH1. 20 patients (14%) received matched therapy, mainly as second-line treatment (45%). The most common matched therapy was an FGFR2 inhibitor (65%). After a median follow-up of 36 months, mOS was 19 months (95% CI, 14-24) in the whole cohort. Difference in mOS was clinically meaningful and statistically significant comparing patients receiving matched treatment to non-matched ones (29 versus 17 months, p = 0.043) (Figure).

**Conclusion:** In our cohort, the use of molecularly matched treatment was associated with significantly longer mOS. Molecular profiling in BTC is crucial to improve treatment strategy and patients' outcomes and should be routinely performed in clinical practice.

**Figure:**


Matched therapy 0 1
p = 0.043
Survival Probabilities
1.00
0.75
0.50
0.25
0.00
0
25
50
75
100
125
Time (months)

# POSTER ABSTRACT PRESENTATIONS

# Nurses & AHPs

# PO1-07
# Integrated hepatitis testing and treatment intervention among people who inject drugs: a comprehensive approach to increase linkage to care towards prevention of progression to hepatocellular carcinoma

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**Background and aims:** Hepatocellular carcinoma (HCC) is the most common primary liver malignancy, the 6th most prevalent cancer and the second most frequent cause of cancer-related death globally. WHO estimates that in 2015, 257 million people were living with chronic Hepatitis B Virus (HBV) infection worldwide, and that 900 000 had died from HBV infection, mostly as a result of cirrhosis or HCC. People who inject drugs (PWID) are a high-risk group for viral hepatitis B and C infections and its accelerated progression to HCC due to their lifestyle. we carried out a community-based hepatitis interventional study integrated into an existing HIV prevention and care program among PWID in 43 locations in Taraba state Nigeria to determine the effect of drug use in increasing vulnerability to the transmission of Viral Hepatitis and the effectiveness of early detection in slowing progression of the diseases and risk of liver cancer among PWID.

**Method:** To reach this often marginalized and hard-to-reach population, we included in the study adult 680 individual aged 18- 55 we excluded children 17 years and below. This was an 18 months study from Feb 2022 to June 2023. WHO PQ rapid test kits was used to screening for HBsAg and Anti-HCV. The test was carried out in the PWID community referred to as bunks, including temporary shelters. 17 individuals were reactive for HBsAg, and 13 for Anti-HCV, no coinfection. Viral load (VL) samples were collected to confirm viraemia for Hepatitis C treatment and to determine eligibility for Hep B treatment in line with the WHO guideline. Simple percentage and linear regression were employed as suitable statistical tools.

**Results:** Of the 17 (PWID) with reactive HBsAg, 12 had viral loads ranging from 4, 000 IU/ml to 5, 000 IU/ml, while 5 had VL exceeding 7, 000 IU/ml. All 13 PWID with reactive Anti-HCV had VL within the range of 7, 000 IU/ml to 11, 000 IU/ml, indicating active viral replication. 15 of the 17 individual commenced treatments with Tenofovir (TDF) for Hep B, 2 were lost to follow-up while all 13 PWID are eligible and commenced DAAs treatment for Hep C alongside other medication regimens and supportive care.All patients for TDF and DAAs were more of male between 25 to 45 years, with 73% male and 27% female. TDF and DAAs treatment between 3 to 6 months shows a significant decline in VL and Alanine Aminotransferase levels. 12 of the 13 individuals who completed DAA treatment has Sustained Virologic Response showing an undetectable HCV RNA level 12 weeks after stopping antivirals.

**Conclusion:** Our integrated approach to hepatitis testing and treatment among PWID in community settings proved effective in reaching a vulnerable population at high risk of viral hepatitis infections as well as HCC. Identifying a substantial number of individuals with active HBV and HCV infections underscores the urgent need for tailored interventions within this population.

# PO5-08-YI
# Improvement of quality of care and patient satisfaction for patients with hepatocellular carcinoma via implementation of a specialist nurse-a pilot study

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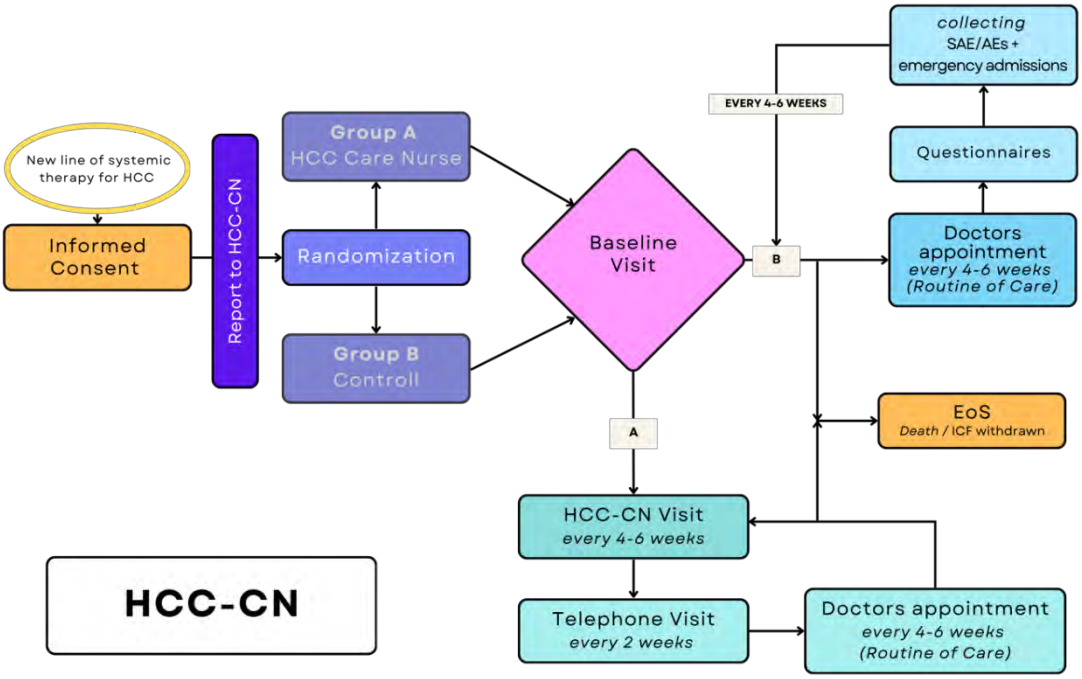
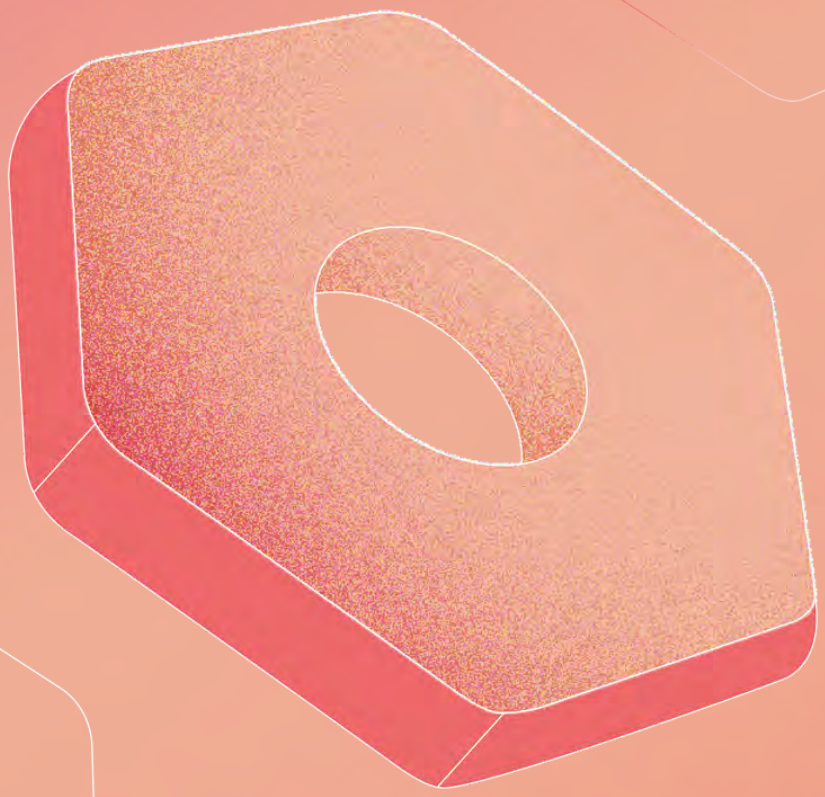
**Background and aims:** Care nursing provided by specifically trained personnel manages the complex interplay between the patient and different medical disciplines. Implementing a hepatocellular carcinoma (HCC) care nurse (HCCCN) has already been shown to be beneficial and cost effective and to reduce adverse events in patients with HCC treated with sorafenib. While the number of patients with HCC is high and even increasing in tertiary care centres, care nursing is not broadly available in Austria. The primary study aim is to standardise patient care in a large tertiary HCC care centre in Vienna, Austria and to implement an HCCCN in daily clinical practice. We will evaluate the implications of this implementation on quality of life, incidence of emergency admissions as well as length of hospitalisation, occurrence and severity of adverse events and time on systemic treatment in patients with HCC.

**Method:** This study will be conducted as a single centre, prospective, pilot-study. All consecutive patients with HCC treated with systemic therapy at the Medical University of Vienna will be evaluated for eligibility to be enrolled in this study. Patients will be 2:1 randomised into the intervention arm (HCCCN) and the control arm (no HCCCN; 40 in the intervention and 20 in the control arm planned).
In addition to quality-of-life questionnaires, we will implement a self-developed patient satisfaction questionnaire to test for the primary study aim. At each visit, patients will fill out the questionnaires and be seen by the treating physician, as well as by the HCCCN for the intervention arm. Additionally, in the intervention arm, telephone visits will be performed by the HCCCN every two weeks, and a pre-standardised checklist will be filled-out.

**Conclusion:** Implementation of an HCCCN may allow for more efficient management of HCC patients in tertiary care centres, and also empower the patient to manage his or her disease more effectively. On top, implications on adverse events, time on treatment and ultimately, survival benefits have yet to be explored. This prospective pilot-study will provide high-quality data on the implementation of an HCCCN.

**Figure:**


New line of systemic therapy for HCC
Informed Consent
Report to HCC-CN
Group A
HCC Care Nurse
Randomization
Group B
Controll
Baseline Visit
EVERY 4-6 WEEKS
collecting
SAE/AEs +
emergency admissions
Questionnaires
B
Doctors appointment
every 4-6 weeks
(Routine of Care)
EoS
Death / ICF withdrawn
A
HCC-CN Visit
every 4-6 weeks
Telephone Visit
every 2 weeks
Doctors appointment
every 4-6 weeks
(Routine of Care)
HCC-CN



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